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A STUDY OF CORNIFICATION IN VAGINAL, BUCCAL AND URINARY SEDIMENT SMEARS*

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ABSTRACT

In order to evaluate the usefulness of urinary sediment and buccal mucosal smears in comparison to vaginal smears for the assessment of estrogen effect, epithelial cell counts to determine the percentage of cornification were made daily throughout the menstrual cycle in 5 young women. The percentage of cornified cells found in the urinary sediment smears paralleled that found in the vaginal smears at various phases of the menstrual cycle, whereas the correspondence between vaginal and buccal smears was poor. The presence of estrogen-sensitive cells in the urinary tract was confirmed by study of a young woman with congenital absence of the uterus and vagina but with a known active ovary. In this subject, peaks of cornification were observed in the cells of the urinary sediment in regular association with the mid-cycle shift in basal body temperature. In cases in which vaginal smears are difficult to obtain, urinary sediment is satisfactory for cornification counts.

THE validity of the cornification count as a qualitative indicator of estrogen effect in the vaginal secretions has become firmly estab-

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lished. The capacity of exogenous estrogen to transform the atrophic cells in the vaginal smears of postmenopausal women into well cornified cells was shown in a classic study by Papanicolaou and Shorr (1). Systematic variations in the proportion of the various types of epithelial cells and a tendency for the cornified cells to reach a maximum (the "cornification peak") near the time of ovulation were found to occur during the menstrual cycle of primates (2, 3). More recently, the cornification peak has been shown to coincide with a peak in the urinary excretion of estrone (4).

The assessment of estrogen effect by differential counting of the epithelial cells in vaginal smears has proved to be a satisfactory clinical procedure in the majority of patients. However, in certain subjects (children and young adolescents) and in patients with pelvic cancer who have been subjected to exenteration operations, vaginal smears are inconvenient or impossible to obtain. In these instances, supplementary methods are required. It was the purpose of this study to evaluate the potential usefulness of smears of the urinary sediment and buccal mucosa in subjects presenting these special difficulties regarding procurement of vaginal smears.

According to a number of reports, the epithelia of the mouth and urinary tract constitute additional sources of estrogen-sensitive cells. Ziskin and Moulton (5) noted some parallelism between the degree of cornification in oral and vaginal smears during the menstrual cycle and during estrogen therapy in deprivation states.

Cifuentes Delatte (6) observed zones resembling vaginal epithelium near the trigone of the bladder. Del Castillo *et al.* (7) described the morphology and the proportions of cells in the urinary sediment during the various epochs of reproductive life and pointed out their resemblance to vaginal cells. Using specimens collected by means of a catheter introduced only into the meatus of the bladder and rinsing the anterior urethra with a drop of glycerin, these investigators demonstrated that the urinary cells may arise from the urethral epithelium. Although they published illustrations showing the resemblance of simultaneously obtained urinary and vaginal smears, they did not record the day-to-day changes in the proportions of the different cell types. Solomon *et al.* (8) used the cells of the urinary sediment as an indicator of hormone levels in pregnancy and in senile urethritis. They postulated that since both the vaginal epithelium and that of the urethra and trigone are derived from the urogenital sinus, they are similar in structure and in their responsiveness to hormonal stimulation.

We have investigated the extent of correlation between the proportions of epithelial cell types in simultaneous buccal, vaginal and urinary sediment smears of young women throughout the menstrual cycle. Supplementary evidence concerning the presence of estrogen-sensitive cells in

the urinary tract was also obtained by study of a young woman with congenital absence of the uterus and vagina.

METHODS

Five young female volunteers provided simultaneous vaginal, buccal and urinary sediment smears. An attempt was made to obtain these daily throughout 2 menstrual cycles (in 1 subject, only 1 cycle was studied) but occasionally specimens were not procured, especially on weekends. Vaginal fluid was taken from the posterior fornix in the usual manner. Oral smears were prepared by scraping the mucosa lining the cheek with a tongue depressor and spreading the material on slides. Urine was obtained by having the subjects void into a clean bottle after they had carefully washed the perineum. One woman occluded the vagina with a tampon before passing urine as an additional precaution against contaminating it with vaginal cells. For each subject, a 25-ml. sample of urine was centrifuged for fifteen minutes at 1500 rpm and the sediment spread on a slide. All specimens were fixed in 95 per cent alcohol and stained by the Papanicolaou method.

Three types of cells were counted:

1. Cornified superficial cells:—nuclei pyknotic; cytoplasm red or pink, sometimes light blue; outline angular.
2. Precornified (intermediate) cells:—nuclei vesicular; cytoplasm light or darker blue or greenish; outline angular.
3. Basal cells:—nuclei vesicular and large; cytoplasm blue; shape oval.

In practice only cornified and precornified cells were recorded, as in these young women there were only 0 to 2 per cent basal cells. Two hundred epithelial cells were counted, 100 from each of 2 different areas of the slide.[1] The percentage of cornified cells recorded was the arithmetic mean of the 2 counts.

Medical histories were taken and both general physical and pelvic examinations were performed on the 5 subjects. All were found to be in good health with no evidence of gynecologic disease.

Subject A. Single, age 30, para 0, gravida 0, menses began at age 12, regular.

Subject B. Married, age 22, para 0, gravida 0, menses began at age 12, regular. (Pregnancy occurred five months after this study.)

Subject C. Single, age 25, para 0, gravida 0, menses began at age 12, regular.

Subject D. Single, age 20, para 0, gravida 0, menses began at age 12, regular.

Subject E. Married, age 23, para 1, gravida 1, menses began at age 13, regular. The cycle studied occurred six months after delivery.

In addition we studied a young woman (*Subject F*) with a congenital anomaly which rendered her peculiarly suitable for this investigation. She had first sought medical attention at the age of 18 because she had never menstruated. Breast development had begun when she was 12 years of age, pubic and axillary hair growth had followed, and she regularly experienced mood swings which her mother likened to premenstrual tension. She exhibited feminine rounding of the figure, slight facial acne, breast development to the secondary mamma stage and normal sex hair growth. A rounded, slightly tender mass was palpable in the left inguinal region. The clitoris and urethral meatus

[1] A statistical analysis of this method of counting, with tables of standard deviations, is given in an article by Salinger, S. L.; Weber, A. A., and Borth, R.: Proliferative effect of testosterone propionate on human vaginal epithelium, *Acta endocrinol.* **4**: 265, 1950.

presented a normal appearance but the vaginal introitus was absent. On rectal examination a rounded mass, which x-ray studies revealed to be a solitary pelvic kidney, was palpable anterior to the sacrum. Cytologic studies of buccal smears showed the sex chromatin to be female in character. At exploratory laparotomy, no uterus or vagina could be found. A left indirect inguinal hernia contained a vestige of Fallopian tube, a small nodule of smooth muscle (probably a remnant of the uterus), and an immature ovary on a long pedicle. A normal-sized active ovary was seen high in the right pelvis. Because of the possibility that torsion of the left ovarian pedicle might occur, all of the contents of the sac were excised prior to repair of the hernia.

Seven months postoperatively, at age 19, this patient volunteered to cooperate in a study to determine whether cyclic changes occurred in the epithelial cells of the urinary sediment. She kept early morning temperature records throughout the period of study.

RESULTS AND DISCUSSION

Curves of the percentage of cornified cells in the vaginal, buccal and urinary smears were plotted for each of the 9 cycles studied. An example is shown in Figure 1. Six of the cycles (Subject A, 1 and 2; Subject B, 1; Subject C, 1 and 2; Subject E) appeared to be ovulatory in character. The vaginal smears showed a relatively low level of cornification at the beginning of the cycle, an intermenstrual peak of cornification with a clean ovulatory field and, in 3 instances, a postovulatory peak. Progestational changes (clumping and curling of the cells) were observed in all of these 6 cycles. Of the other 3 cycles, 1 was definitely anovulatory (Subject D, 1) and 2 were doubtful in character (Subjects B, 2 and D, 2). Although in the first cycle of Subject B the smear was typically ovulatory, in the second cycle it showed marked cytolysis and absence of progestational changes. In Subject D there was a relatively high level of cornification throughout

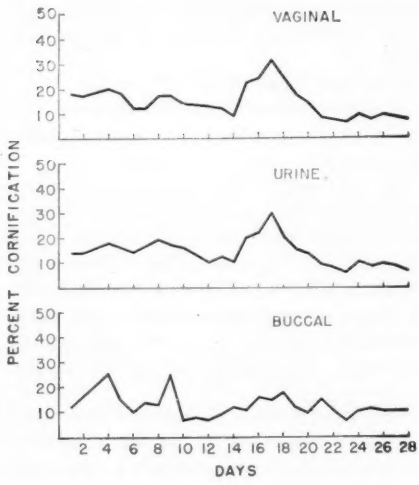

FIG. 1. Cornification peak on the seventeenth day in both vaginal and urinary curves; no peak in the buccal curve. (Subject E, who used vaginal tampons when obtaining a specimen of urine.)

the first cycle, with irregular peaks up to 78 per cent cornified cells, and no progestational effects; in the second cycle also, there were high percentages of cornified cells (especially during the second half) but only slight clumping and curling.

In accordance with the observations of others (9), we found that the actual percentage of cornified cells was not as important an indicator of ovulation as the shape of the curve. The maximum varied from 31 to 87 per cent, and the minimum from 3 to 20 per cent (Table 1). It is of interest that in each subject the level of cornification in the 2 cycles was remarkably constant, and that in 2 women the peak scarcely exceeded 30 per cent.

In the urinary sediment smears both the maximum and minimum percentages of cornified cells were slightly lower than in the vaginal smears (Table 1). Progestational changes could not be definitely determined from urinary smears. In Subject F, who had no Müllerian duct structures, 4 peaks of cornification occurred in the urinary cells during the 16-week period of study. The percentage of cornified cells varied between a maximum of 26-35 per cent and a minimum of 2 per cent. These levels corresponded to those observed at the peak in 2 of the other subjects. The shape of the curves in Subject F was ovulatory and the peaks slightly preceded

TABLE 1. PERCENTAGE OF CORNIFIED CELLS IN SIMULTANEOUS SMEARS DURING MENSTRUAL CYCLES

	Vaginal smear		Urinary smear		Buccal smear	
	Max.	Min.	Max.	Min.	Max.	Min.
A.	60	13	60	15	34	5
	63	19	48	18	28	11
B.	32	3	25	8	28	9
	33	6	29	6	41	13
C.	74	20	64	15	44	14
	87	15	81	16	40	13
D.	76	13	45	5	29	7
	78	9	66	8	28	10
E.	31	6	30	6	26	6
F.			26	2		
			35	3		
			28	3		
			33	3		

the luteal rise in basal body temperature. Two representative cycles are shown in Figure 2.

In the buccal smears, the maximum percentage of cornified cells varied from 26 to 44 per cent, and the minimum from 6 to 18 per cent. The high points did not always correspond to those in the vaginal smears. We thought, therefore, that it would be of interest to determine the degree of correlation between the buccal and vaginal, and the urinary and vaginal counts.

The product moment method was used to compute the coefficient of correlation, which is shown for all curves in Table 2. A strongly positive correlation was obtained between vaginal and urinary cornification, the coefficients ranging from +0.72 to +0.95. The correlation between vaginal and buccal cornification, on the other hand, proved to be poor, the range in 8 of the 9 instances varying from −0.39 to +0.57. In 1 instance (Subject C, 1) it reached +0.725, a magnitude similar to that found in the vaginal-urinary correlations. In this woman (C), the percentage of cornified cells in the vaginal and urinary smears reached a high peak in both cycles, but the percentage was high in the buccal smear in only one cycle. The close correlation between vaginal and urinary counts was maintained even in the subjects in whose cycles no definite intermenstrual peak of cornification was observed.

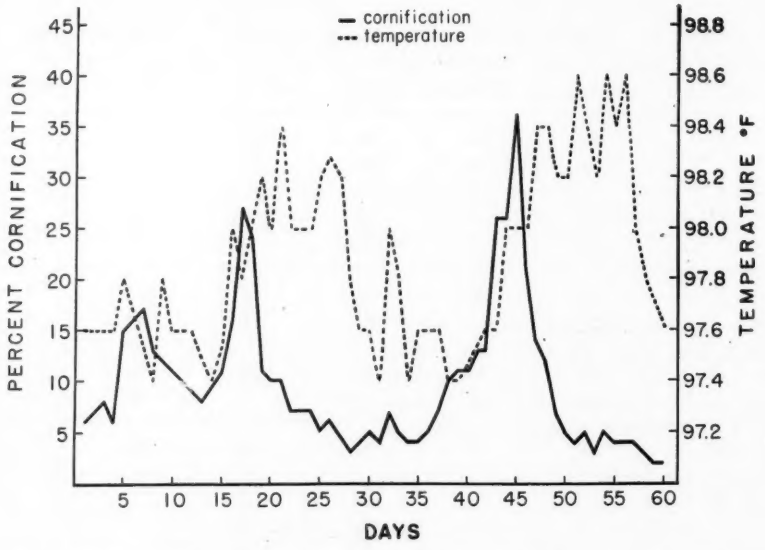

FIG. 2. Curve of cornification of urinary cells throughout 2 menstrual cycles. Basal temperature curves are superimposed. (Subject F, who had no uterus or vagina.)

TABLE 2. COMPARISONS OF CYTOLOGIC CURVES

The vaginal-urinary relationship is statistically significant in all curves.

Subject & Cycle	Vaginal-Urinary			Vaginal-Buccal		
	N*	r	P less than	N*	r	P less than
A I	19	+0.716	0.001	26	+0.333	0.1
II	17	+0.716	0.001	26	+0.284	0.2
B I	13	+0.887	0.001	20	+0.516	0.02
II	12	+0.856	0.001	23	+0.576	0.01
C I	19	+0.818	0.001	24	+0.725	0.001
II	12	+0.958	0.001	21	+0.413	0.1
D I	20	+0.917	0.001	26	−0.188	0.5
II	17	+0.892	0.001	26	−0.398	0.05
E	26	+0.893	0.001	26	+0.261	0.2

* N is the number of observations, r is the correlation coefficient, and P is the probability, obtained by using the Student "t" test.

The morphologic resemblance between the cells of the urinary sediment and those of the vaginal fluid, and the strong positive correlation between their cornification counts raised the question as to whether, in spite of the special precautions taken, the urine had been contaminated with vaginal cells. The fact that the urinary sediment smears obtained when the vagina was occluded with a tampon (Fig. 1) were similar to those obtained without this added precaution suggests that the cells did not arise from the vaginal tract. Conclusive proof of the urinary origin of these cells was obtained in Subject F, in whom the urinary tract was their only possible source.

Acknowledgments

We wish to thank Dr. Mindel Sheps for her invaluable help with the statistical analyses, and Dr. E. B. Taft and Dr. Howard Ulfelder for their help and advice.

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EFFECTS OF INTRAVENOUS INFUSIONS OF *dl*-ALDOSTERONE ACETATE ON SODIUM AND POTASSIUM EXCRETION IN MAN*

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ABSTRACT

Intravenous infusions of *dl*-aldosterone monoacetate in 5 per cent glucose-water were given at a rate of 4.2 μg. per minute over an eight-hour period to 3 patients with Addison's disease who received two-hourly feedings during the 24-hour period of study. Urine collections were made at two-hourly intervals. Following the commencement of the infusions two hours elapsed before an appreciable effect on sodium and potassium excretion was observed. Sodium antidiuresis and potassium diuresis then occurred and lasted four to six hours after stopping the infusion. No consistent effect on salivary Na/K ratio or on circulating eosinophils was noted. The simultaneous infusion of 20 mg. of hydrocortisone over the eight-hour period did not modify the effect of aldosterone on sodium excretion but caused an increase in potassium diuresis. Serum sodium and potassium concentrations both fell during the period of study.

THE availability of synthetic *dl*-aldosterone monoacetate for use in clinical research enables an evaluation to be made of the physiologic properties of this steroid in man. Since supplies were extremely limited, the present study was confined to observations of the immediate effects on the excretion of sodium and potassium in the urine and saliva. Infusions of the compound were given over periods of eight hours to patients with Addison's disease maintained on the day of the infusion, and for the previous two days, with sodium chloride. In particular, the time relationships of the effects of aldosterone on the handling of sodium and potassium by the renal tubule were investigated, since Barger, Berlin and Tulenko (1,

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2) have reported a delay of thirty to sixty minutes before an effect on sodium and potassium excretion was observed in unanesthetized dogs when *d*-aldosterone-21-ol was injected directly through a catheter into the renal artery. Ganong, Mulrow and Hollinger (3), using a similar experimental preparation, reported a delay of five to thirty minutes before an effect on sodium and potassium excretion became apparent. Barger and co-workers (1, 2) also noted that the infusion of *d*-aldosterone-21-ol (10 μg. over a period of two hours) into the renal artery of a normal animal was without effect in the dosage used, whereas the same dose in the adrenalectomized animal caused potassium diuresis and sodium antidiuresis. The possibility that this could be explained on the basis of competition between steroids was investigated by a comparison of the result of an infusion of aldosterone alone with that of the simultaneous infusion of aldosterone and hydrocortisone.

METHODS AND MATERIAL

These studies were carried out in the Metabolic Ward of the Peter Bent Brigham Hospital. For two days before the day of the infusion the patients received a constant diet containing 180 mEq. of sodium and 150 mEq. of potassium. No steroid therapy was given during this period.

Since the taking of normal meals during the period of the infusions would throw a fluctuating load of sodium on the kidneys, and since it was deemed inadvisable to starve patients with Addison's disease for the duration of the study, each patient was given two-hourly feedings at 8 A.M., 10 A.M., 12 noon, 2 P.M., 4 P.M., 6 P.M., 8 P.M., 10 P.M., 12 midnight and 6 A.M. The composition of these feedings is given in the legends to the individual figures.

Urine was collected at two-hour intervals throughout the twenty-four hours, with the exception of the period midnight to 6 A.M. Saliva was collected by giving the patients paraffin wax to chew, discarding the secretion during the first ten-minute period and retaining that secreted during the second ten-minute period. Its volume and sodium and potassium concentration were measured. Eosinophils were counted by the method of Fisher and Fisher (4). Chloride was estimated by the method of Schales and Schales (5), sodium and potassium concentrations were determined by flame photometry, and 17-hydroxycorticoids were measured by the method of Reddy (6).

The patients were kept in bed throughout the 24-hour period of study to minimize alteration of renal blood flow with change of posture. Control infusions of 5 per cent glucose-water or of hydrocortisone in 5 per cent glucose-water were given in all cases to determine the diurnal variation of sodium and potassium excretion in these patients under the conditions of the experiment. Blood pressure readings were made at two-hourly intervals throughout the twenty-four hours. Serum sodium and potassium concentrations were determined at intervals throughout the period of study, as was the hematocrit.

Patient M.P. was a 68-year-old male with a history suggestive of Addison's disease of six months' duration. He was admitted for diagnosis and has never received steroid treatment before the infusion was given, with the exception of 1 mg. of fluorohydrocortisone daily during a three-day corticotropin test. His basal excretion of 17-hydroxy-

corticoids was 5.2 mg. per twenty-four hours but showed no increase during a three-day infusion of corticotropin.

Patient W.G. was a 40-year-old male with Addison's disease of seven years' duration, normally maintained with cortisone (12.5 mg. daily) and fluorohydrocortisone (0.2 mg. daily). His basal excretion of 17-hydroxycorticoids was 1.7 mg. per twenty-four hours and showed no response to corticotropin.

Patient R.S. was a 58-year-old female with Addison's disease of fifteen years' duration, normally maintained with cortisone (37.5 mg. daily) and deoxycorticosterone trimethylacetate (25 mg. monthly intramuscularly). Her basal excretion of 17-hyroxycorticoids was 1.6 mg. per twenty-four hours and showed no response to corticotropin.

dl-Aldosterone monoacetate dissolved in ethanol was diluted to 400 ml. with 5 per cent glucose solution and given as a constant intravenous infusion over eight hours from 10 A.M. to 6 P.M. An infusion of 2 mg. over eight hours is a rate of 4.2 μg. per minute of the monoacetate of the racemic compound, equivalent to 1.94 μg. per minute of *d*-aldosterone as the free alcohol. The amount of free aldosterone secreted by the adrenal cortex under normal conditions has been estimated by Ayres and associates (7) to be 190 μg. per day, or 0.14 μg. per minute.

RESULTS

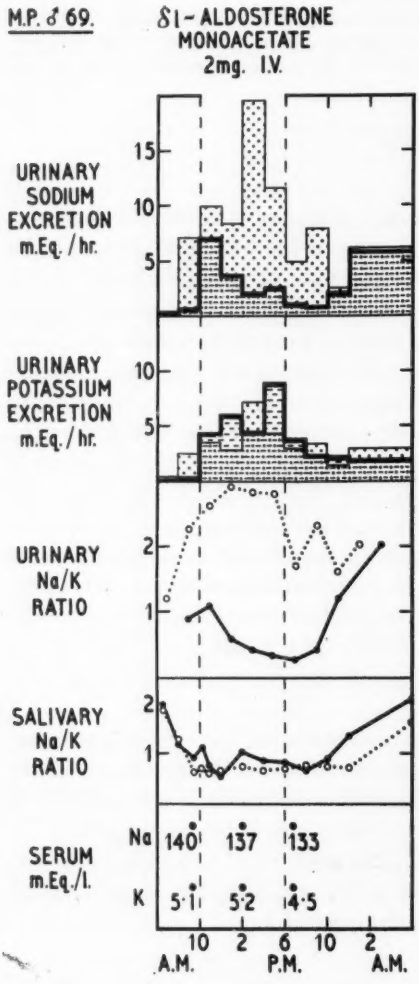
Infusion of dl-aldosterone monoacetate

(a) *Effect on renal excretion of sodium, potassium and chloride.* In all 3 patients the amount of sodium excreted in the urine was decreased, and the amount of potassium increased, during, and for two to six hours following the period of the infusion of *dl*-aldosterone (Figs. 1–3). The concentration of sodium in the urine decreased, and that of potassium increased. In no case was an appreciable effect demonstrable during the first two-hour period of the infusion. The maximum depression of the urinary Na/K ratio was observed at the eighth to tenth, or tenth to twelfth hours after the commencement of the infusion, that is, two to four hours after the infusion had ceased. In Patients W.G. and R.S. the effect of the hormone on the excretion of potassium was appreciable but perhaps not as great as that on the excretion of sodium; in Patient M.P. the effect on potassium excretion was not nearly as pronounced as that on sodium excretion. The effect of the infusion of 1 mg. of *dl*-aldosterone acetate into Patient M.P. under identical conditions was found to be as effective as that of 2 mg. of this steroid. No effect on the excretion of chloride was noted during the infusion of aldosterone into this patient.

(b) *Effect on the salivary Na/K ratio.* A fall of the salivary Na/K ratio during and following the infusion was noted in all cases. However, when the curve obtained on the days when aldosterone was given is compared with the control curve there is no appreciable difference between the two in the case of Patient M.P.; in Patient W.G. the ratio on the experimental day lies below that of the control day, but in Patient R.S. the ratio when aldosterone was given lies above that of the control day.

Modification of the effect of aldosterone on sodium and potassium excretion by the simultaneous infusion of hydrocortisone

Infusion of hydrocortisone alone into Patients R.S. and W.G. caused a lowering of the Na/K ratio, due entirely to an increased excretion of potassium. When the 2 steroids, hydrocortisone and aldosterone, were infused simultaneously there resulted an increased excretion of potassium, compared with the occasion when either aldosterone or hydrocortisone was given alone (Fig. 4). Retention of sodium occurred to the same extent when the 2 steroids were given simultaneously as when aldosterone was given alone.



Other observations

No consistent effect on the eosinophil count was noted. No alteration of blood pressure occurred during the experimental period. Serum sodium and serum potassium concentrations fell during the course of the infusion in all cases. No weight gain was observed. In 1 case (M.P., Fig. 1), the commencement of the infusion corresponded to a great increase in urine output (from 17 ml. to 370 ml. over the two-hour period), but during the control infusion of 5 per cent glucose in water a similar, but not as pronounced a rise of urine volume occurred at the commencement of the infusion.

FIG. 1. Effect of the intravenous infusion of *dl*-aldosterone monoacetate (2 mg. during eight hours) on the urinary excretion of sodium and potassium and on the urinary and salivary Na/K ratios in Patient M.P. (Results obtained on the control day, when an infusion of 5 per cent glucose-water was given, are represented by the dotted area; those obtained on the experimental day are represented by the dashed area). Two-hourly feedings contained 21 mEq. of Na, 15 mEq. of K, and 350 ml. of water. Total calories = 335.

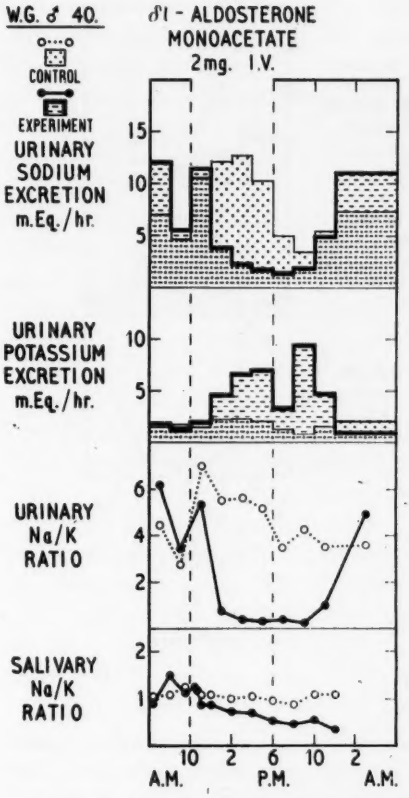


FIG. 2. Effect of the intravenous infusion of *dl*-aldosterone monoacetate (2 mg. during eight hours) on the urinary excretion of sodium and potassium in Patient W.G. Two-hourly feedings contained 14 mEq. of Na, 10 mEq. of K, and 300 ml. of water. Total calories = 280.

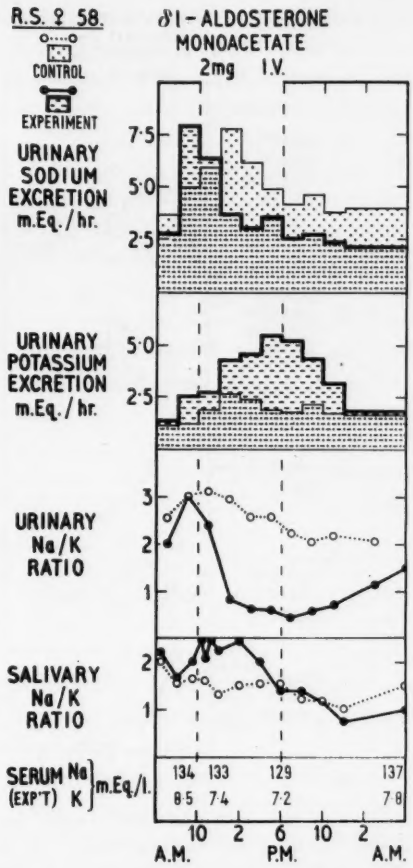


FIG. 3. Effect of the intravenous infusion of *dl*-aldosterone monoacetate (2 mg. during eight hours) on the urinary excretion of sodium and potassium in Patient R.S. Two-hourly feedings contained 20 mEq. of Na, 15 mEq. of K, and 300 ml. of water. Total calories = 300.

DISCUSSION

These findings confirm previous observations that the short-term administration of aldosterone is followed by a decrease in the renal excretion of sodium and an increase in the renal excretion of potassium without any consistent effect of urine volume, and (in the single case in which it was measured) with no effect on the excretion of chloride.

The mechanism whereby adrenocortical steroids modify the renal han-

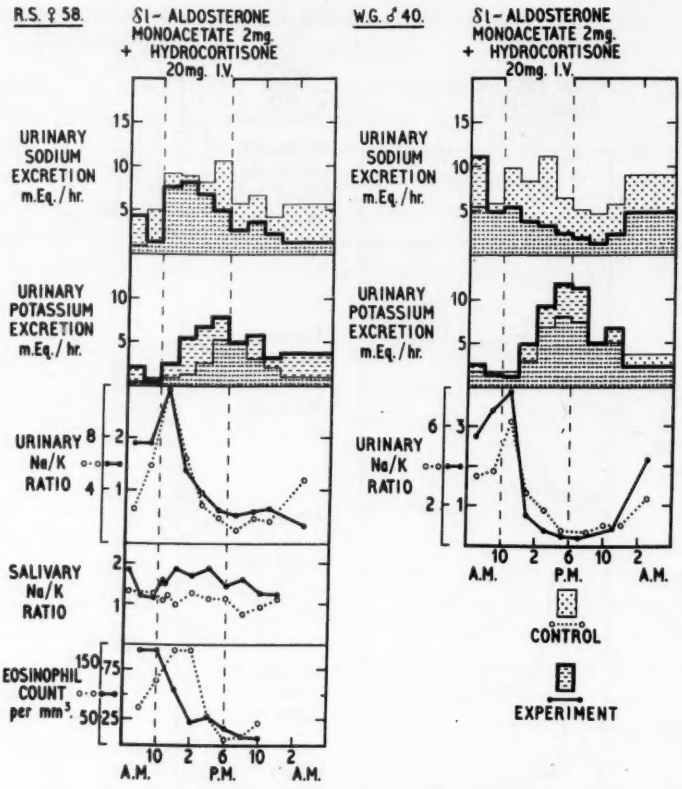


FIG. 4. Effect of the simultaneous intravenous infusion of *dl*-aldosterone monoacetate (2 mg.) and hydrocortisone (20 mg.) during an eight-hour period in Patients R.S. and W.G. The control infusion was of hydrocortisone (20 mg.) in 5 per cent glucose-water.

dling of sodium and potassium is not known and hence no convincing explanation is possible of the surprising finding of a delay of two hours before an effect on the excretion of these electrolytes becomes apparent.

In the experimental dog, Barger and colleagues (1, 2) showed that the delay is identical irrespective of whether the hormone is injected into a peripheral vein or directly into the renal artery. A similar delay is seen in the graphs of Thorn and associates (8), not only for the infusion of *d*-aldosterone-21-ol (200 μg. over a period of four hours) but also for the infusion of 9α-fluorohydrocortisone into patients with Addison's disease. It is possible that these substances may be changed into a different chemical form before they are capable of action on the renal tubule. Another possi-

bility is that the delay is due to the slow adaptation of the responsible enzyme in an electrolyte transport system to the presence of the steroid, so that some time elapses before this hypothetical enzyme is present in amounts adequate to produce a measurable alteration in urinary electrolyte concentration.

It is evident from the existence of a delay period that the secretion of aldosterone does not constitute a mechanism for the control of sodium reabsorption in response to minute-to-minute fluctuations of sodium load. However, it does not rule out the possibility that the presence of aldosterone is required for the maintenance of this mechanism.

The intravenous infusion of hydrocortisone under identical conditions results in an increase in urine flow and in an increased excretion of sodium without an increase in sodium concentration. The Na/K ratio in the urine fell during the administration of hydrocortisone in a manner quite similar to that occurring during the infusion of aldosterone, but for a different reason. When hydrocortisone was infused the Na/K ratio fell because of a five-fold increase in the amount of potassium excreted; the amount of sodium excreted in fact rose, rather than fell, during the infusion.

No evidence of competition was found when aldosterone and hydrocortisone were infused simultaneously. The effect on sodium excretion when both hormones were infused was almost identical with that when aldosterone was given alone. The excretion of potassium, however, was much greater when both hormones were given together than when aldosterone was given alone. This observation may have some bearing on the potassium wasting that occurs in cases of Conn's syndrome.

An infusion of *d*-aldosterone as the free alcohol at a rate of 0.83 μg. per minute had been given to Patient W.G. two years previously (Thorn *et al.* (8)). The effect of this infusion on the Na/K ratio was almost superimposable upon that produced by the infusion of *dl*-aldosterone acetate at a rate of 4.2 μg. per minute (equivalent to 1.94 μg. per minute of free *d*-aldosterone). It is thus apparent that a maximum effect had been obtained with a dosage of 0.83 μg. per minute and it is probable that the minimum effective amount is lower than this. At this dosage level, *d*-aldosterone was equally as effective in lowering the Na/K ratio in the urine as was 9α-fluorohydrocortisone, weight for weight (Thorn *et al.* (8)).

A fall of serum sodium concentration was consistently observed during the infusions of aldosterone. Since sodium retention was occurring during, and for two to four hours after the infusion, and no change in hematocrit was observed, this fall of serum sodium concentration suggests that sodium was entering cells under the influence of aldosterone. The fall of serum potassium concentration can be explained on the basis of a negative potassium balance during the period of study.

Thorn and co-workers (8) observed a decrease in the circulating eosinophils during prolonged administration of aldosterone in a single patient. However, in the present study with short-term infusions of aldosterone, no consistent effect on circulating eosinophils was observed.

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METABOLISM OF PROGESTERONE-4-C^{14} BY AN ARRHENOBLASTOMA*

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ABSTRACT

Virilizing arrhenoblastoma tissue obtained from a 20-year-old woman was incubated *in vitro* with progesterone-4-C^{14}. Conversion of progesterone to 17-hydroxyprogesterone, androstenedione and at least 2 more polar substances was demonstrated.

ALTHOUGH the ability of ovarian arrhenoblastoma to produce androgenic effects has been recognized for many years, few studies have been made endeavoring to characterize the androgenic substances elaborated by the tumor. An explanation for the associated masculinizing syndrome is that ovarian tumor metabolism results in the relative over-production of androgens. Anliker *et al.* (1) have recently isolated progesterone (31 μg. per Kg.), testosterone (92 μg. per Kg.), 4-androstene-3,17-dione (370 μg. per Kg.) and androsterone (61 μg. per Kg.) from arrhenoblastoma tissue. Previous work (2–6) has associated the biochemical origin of androgenic steroids with the C-21 steroids. Slaunwhite and Samuels incubated progesterone-4-C^{14} *in vitro* with rat testicular tissue and isolated radioactive 17α-hydroxyprogesterone, 4-androstene-3,17-dione, and testosterone (2).[1] This observation has been substantiated and extended in other laboratories (3, 4, 5, 7). Incubations of bovine ovarian tissue have also shown the conversion of progesterone to androgens (6), and Zander has isolated 17-hydroxyprogesterone and androstenedione from normal human corpora lutea (8). Savard *et al.*, in a preliminary report, have shown the conversion of progesterone to 17-hydroxyprogesterone and androstenedione by arrhenoblastoma tissue (9). We are reporting an additional study of the metabolism of progesterone-4-C^{14} by an arrhenoblastoma.

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[1] 17α-Hydroxyprogesterone = 17-hydroxyprogesterone. 4-Androstene-3,17-dione = androstenedione.

CASE REPORT

The patient was a 20-year-old unmarried female. She was first seen in September 1957 with complaints of amenorrhea and increasing hirsutism. Her menstrual cycles had begun at age 13 and were regular until age 18, when, during the next year, she had only three or four widely spaced menstrual periods. For fourteen months prior to September 1957 there had been complete amenorrhea except on two occasions when she bled following the administration of estrogen. The last such bleeding had occurred ten months prior to admission. The patient stated that she had always been somewhat hirsute but had noted an increase in growth of hair on the face, lower abdomen and vulvar region during the previous year; on the face, it had been accompanied by progressing acne.

Physical examination showed normal vital signs. There was moderately severe acne, together with an excessive amount of hair growth on the face. Breast development was within normal limits. There was a slight tendency for the hair growth over the symphysis to extend upward toward the umbilicus. The clitoris was definitely enlarged. The hair growth on the vulva was luxuriant. It extended out on the inner aspect of the thighs and posteriorly to the peri-anal region. Upon pelvic examination, the vagina and cervix were normal. The uterus was in the anterior position, and small. In the region of the right ovary was a moderately firm, well-rounded, mobile mass measuring about 6 cm. in diameter. The left ovary was palpable and smaller than normal.

Routine blood and urine studies yielded normal findings. Urinary 17-ketosteroid excretion values were 11.4 and 11.0 mg. per twenty-four hours. Bioassay for gonadotropin (FSH) demonstrated barely perceptible amounts in the urine. Intravenous pyelograms showed a normally functioning urinary tract.

At laparotomy the right ovary was found to be enlarged (6 cm. in diameter). It was covered by a smooth gray capsule. Small cystic follicles were visible on the surface. On cutting into the mass it was evident that the enlargement was caused by a yellowish fleshy tumor over which was a shell of normal-appearing ovarian cortex. The left ovary was small and atrophic in appearance. It also was covered by a gray capsule. A small wedge-shaped biopsy specimen of this ovary was taken for study. The right ovary was removed, leaving the right tube in place.

Microscopically, the right ovarian tumor was composed of plump fusiform cells. Fine fibrous septa separated these cells into cords and strands, with some tendency toward a tubular arrangement (Fig. 1). Microscopic examination of the left ovary showed follicles in varying stages of development, and the surface covered by a layer of fibrous stromal cells which produced a capsule-like picture (Fig. 2). That this change was a temporary one is indicated by the fact that spontaneous ovulatory cycles were resumed within a month of the removal of the tumor and have recurred at regular intervals since that time.

EXPERIMENTAL

Immediately following excision, the tumor was transferred to an ice-cold receptacle. Within two hours 2 aliquots of tissue weighing respectively 295 mg. and 590 mg. were homogenized in Krebs-Ringer phosphate buffer. Each sample of tissue was incubated with 6 μg. of progesterone-4-C^{14} (400,000 c.p.m.) in 25 ml. of phosphate buffer, pH 7.4, for three and a half hours at 37° C. (Flasks 1 and 2). No co-factors were added to the incubation media.

Steroidal material was subsequently extracted with ethyl acetate, separated from neutral fat (10), and analyzed by paper chromatographic techniques (11). Quantitative estimates of radioactivity on the paper chromatograms were made by scanning the strips with a thin window flow counter equipped with an automatic recording device.

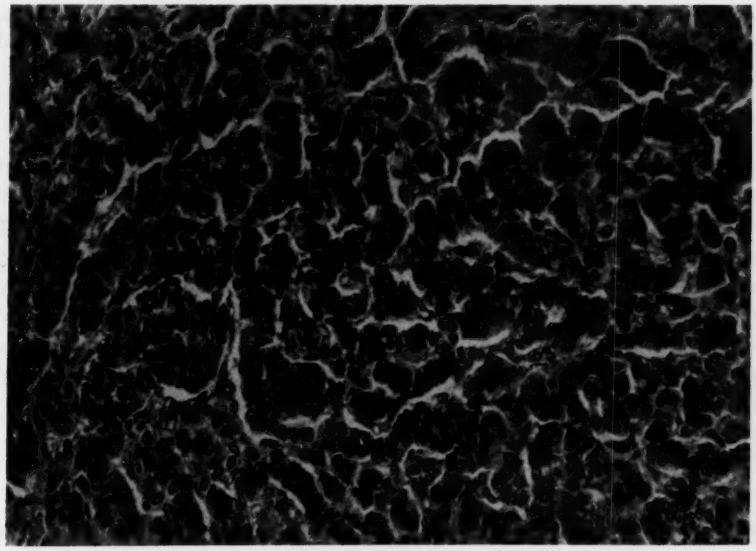

FIG. 1. Microscopic pattern of the tumor. The tumor cells are arranged in cords with little intervening connective tissue. (256×.)

FIG. 2. Histologic section of the biopsy specimen taken from the uninvolved left ovary. The cortex on the surface is made up of closely packed cells producing a "capsule." Primordial follicles are grouped beneath the "capsule." (64×.)

Ultraviolet absorption of carrier steroids used for characterization of the unknown metabolites was measured on the paper chromatograms by means of a Cary recording spectrophotometer operated at 240 mμ (12).

RESULTS

Extracts of the incubated material from both flasks were chromatographed in the heptane-formamide system for six hours. The results were comparable. Those with material from the second flask are shown in Figure 3 (top). Peak III had the same mobility as authentic progesterone and accounted for 31 per cent of the total. Peak II had the same mobility as androstenedione and Δ^4-pregnene-20α-ol-3-one. Three-fourths of Peak I was rechromatographed in benzene-heptane (1:1)-formamide for three and a third hours; the results are shown in Figure 3 (bottom). The chromatographic mobility of Peak I_3 corresponded to that of authentic testosterone and 17-hydroxyprogesterone.

Characterization

The more polar materials, Peaks I_1 and I_2, were not identified. However, both peaks were analyzed by extraction procedures suitable for removing estrogens (13) and by paper chromatography, and were found to be completely free of these steroids. Peak I_2 when chromatographed in benzene-chloroform (4:1)-formamide had an R_f of 0.36. The following authentic steroids were used for comparison in the same system: 11β-hydroxytestosterone, R_f 0.15; 11β-hydroxyandrostene-3,17-dione, R_f 0.50; and 6β-hydroxyprogesterone, R_f 0.55. Addition of authentic testosterone (25 μg.) to an aliquot of Peak I_3 material, followed by acetylation with acetic anhydride in pyridine and chromatography in heptane-formamide for three and a half hours yielded the results shown in Figure 4 (top). Complete acetylation of carrier is evident; however, none of the radioactive material reacted. This demonstrates that Peak I_3 contained no testosterone.

Characterization of Peak I_3 as 17-hydroxyprogesterone is based on the following evidence: a second aliquot was mixed with 50 μg. of authentic 17-hydroxyprogesterone and chromatographed in heptane-benzene (1:1)-formamide (Fig. 4, middle). The fact that the 2 curves can be superimposed demonstrates the homogeneity of carrier with radioactive substance. This mixture, after elution, was oxidized with CrO_3 under mild conditions and rechromatographed in heptane-benzene (1:1)-formamide for two and a half hours (Fig. 4, bottom). Both substances reacted completely, resulting in a product having the same mobility as androstenedione. This material reduced with sodium borohydride yielded a product having the mobility of testosterone, and the latter could be acetylated. Throughout these chemi-

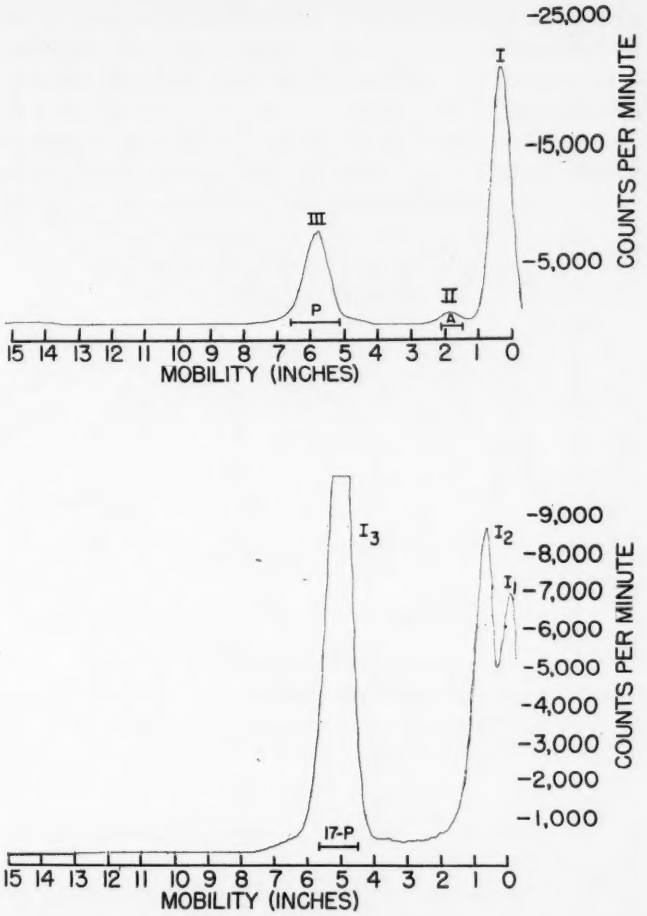

FIG. 3. Radioactivity recording of paper chromatograms. *Top.* Steroid contents of incubation Flask 2 chromatographed in heptane-formamide for six hours. Mobilities of authentic progesterone (P) and androstenedione (A) are indicated by subtending lines. *Bottom.* Peak I material rechromatographed in heptane-benzene (1:1)-formamide for three and a third hours. Mobility of authentic 17-hydroxyprogesterone (17-P) is indicated.

cal manipulations the specific activity of all the products remained constant. Thirty-three per cent of the incubated progesterone was converted to 17-hydroxyprogesterone.

An aliquot of Peak II material was combined with authentic androstenedione and the mixture was acetylated and chromatographed in heptane-

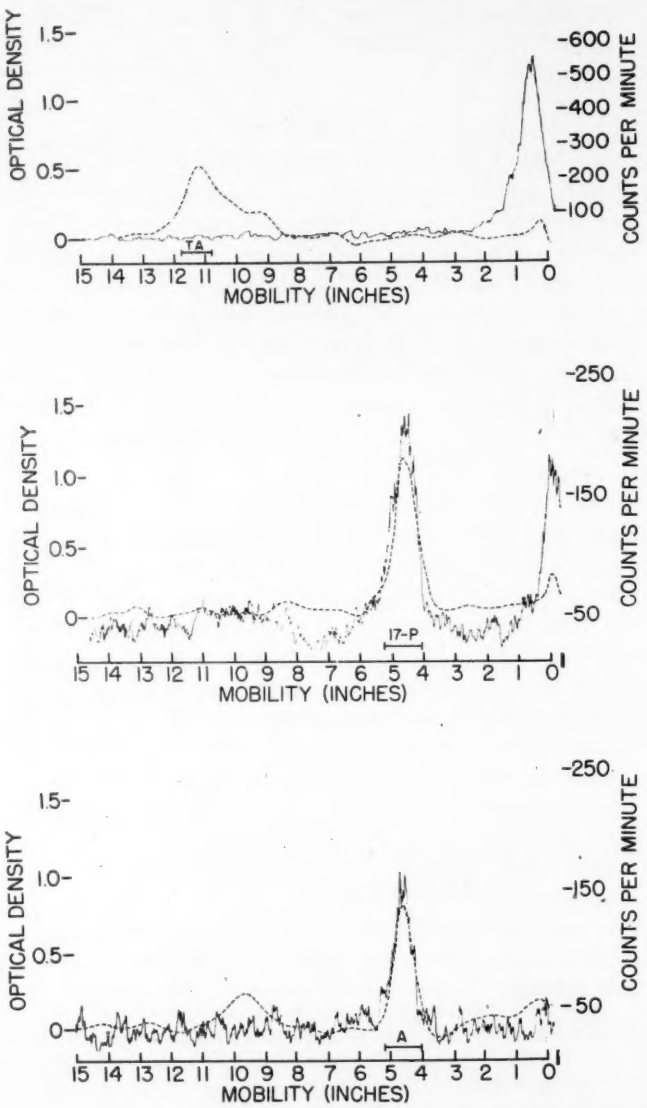

FIG. 4. Radioactivity (solid line) and optical density (dashed line) recordings of paper chromatograms. *Top.* An aliquot of Peak I_3 material plus 25 μg. of testosterone treated with acetic anhydride in pyridine and chromatographed in heptane-formamide for three and a half hours. Mobility of authentic testosterone acetate (TA) is indicated by subtending line. *Middle.* Aliquot of Peak I material plus 50 μg. of 17-hydroxyprogesterone chromatographed in heptane-benzene (1:1)-formamide for four hours. Mo-

formamide for three and a half hours. Peak II material was not homogeneous (Fig. 5), but the largest portion did not become acetylated. Characterization of the nonacetylated portion as androstenedione was obtained by borohydride reduction of eluted carrier and radioactive material to yield a compound having the mobility of authentic testosterone. This reduction product was subsequently acetylated. Of the incubated progesterone, 1.2 per cent was converted to androstenedione.

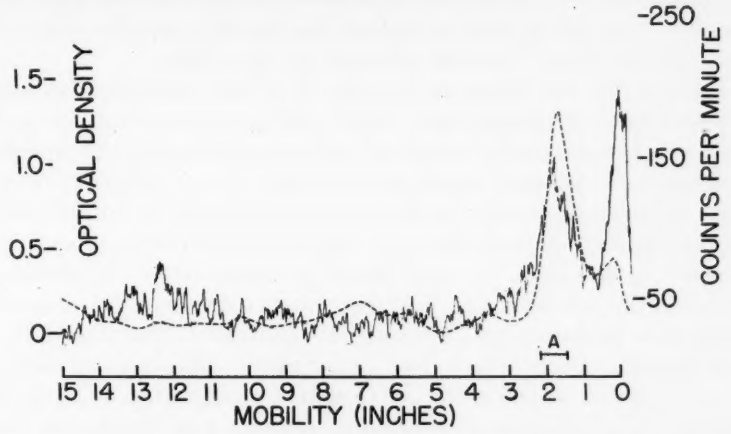

FIG. 5. Radioactivity and optical density recordings. An aliquot of Peak II material plus 50 μg. of androstenedione was treated with acetic anhydride in pyridine and chromatographed in heptane-formamide for three and a half hours. Mobility of authentic androstenedione (A) is indicated.

Figure 5 also shows that part of the Peak II material was acetylated. This acetate had the same chromatographic mobility as 4-pregnene-20α-ol-3-one acetate, and accounted for 0.6 per cent of the incubated progesterone. No 4-pregnene-20β-ol-3-one was detectable in the incubation media.

DISCUSSION

The capacity of tissue from arrhenoblastoma to metabolize progesterone *in vitro* to 17-hydroxyprogesterone, androstenedione and at least 2 more polar substances which are not identified has been demonstrated, and is in

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bility of authentic 17-hydroxyprogesterone (17-P) is indicated. *Bottom.* The material forming the peak in the middle chromatogram was eluted, treated with 4 per cent chromic acid in 90 per cent acetic acid overnight, and chromatographed in heptane-benzene (1:1)-formamide for two and a half hours. Increased optical density at 9.7 inches was due to fluorescence at the solvent front. Mobility of authentic androstenedione (A) is indicated.

agreement with results of Savard *et al.* (9). Testosterone and 4-pregnen-20β-ol-3-one were not formed under our incubation conditions, due possibly to the use of homogenates unfortified with reducing co-factors.

It may be that a similar anabolic pathway (progesterone→17-hydroxyprogesterone→androstenedione) exists under *in vivo* conditions in the tumor. Such a postulate requires the assumption that progesterone is an endogenous constituent of the tumor tissue. The finding by Anliker *et al.* (1) of progesterone in the tumor tissue seems to establish this point in the affirmative. The results of these authors also demonstrate that androstenedione is quantitatively the chief androgen in the tumor.

It appears that the metabolic pattern of arrhenoblastoma with respect to the conversion of progesterone to the androgens does not differ qualitatively from that of normal ovarian tissue. It is possible that the production of androgens in virilizing tumors is accompanied by a diminution in the activity of enzymes involved in the aromatization of ring A and therefore in estrogen production, resulting in a relative overproduction of androgenic hormones. Anliker *et al.* (1) were unable to demonstrate the presence of estrogens in the phenolic fraction of their tumor extracts, and estrogen production from progesterone-4-C^{14} was not observed in our study. In this case virilization would result from quantitative differences in the component enzymes involved in the conversion of progesterone to the ovarian hormones. More information is required before a final conclusion can be drawn.

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THE UPTAKE OF P^{32} AS A MEASURE OF CELLULAR METABOLISM IN THE HUMAN THYROID*†

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ABSTRACT

Cellular metabolism in the thyroid gland of patients with Graves' disease, nodular goiter and thyroid cancer was appraised by measuring the incorporation of radiophosphorus (P^{32}). The P^{32} was administered intravenously twenty-four hours before operation. At the time of surgery, representative specimens were procured from thyroid, muscle and blood. The ratio of counts per minute per milligram of thyroid to counts per minute per .001 milliliter of serum was thought to be the most reliable index of P^{32} content. Representative "thyroid-serum ratios" were as follows: normal thyroid, 23; Graves' disease treated with propylthiouracil and Lugol's solution, 33; Graves' disease treated with propylthiouracil alone, 49; follicular adenoma (nodular goiter), 20; fetal adenoma (nodular goiter), 40; and carcinoma of the thyroid, 71. Administration of propylthiouracil and Lugol's solution to patients with hyperthyroidism seemed to have little demonstrable effect upon the total incorporation of P^{32}. Neither was P^{32} uptake decreased in patients with suppressed hormone synthesis due to inhibition of TSH by *l*-triiodothyronine or desiccated thyroid. The data suggested that the amount of labeled phosphorus incorporated into the human thyroid at twenty-four hours is related more directly to cell density than to functional activity of the gland as judged by histologic criteria.

THE study of cellular metabolism in the normal thyroid gland has been approached principally through the avenues of iodine turnover and hormone synthesis. Such studies have disclosed that the microscopic anatomy is usually, but not always, a reliable index of metabolic activity (1). Most diseases of the thyroid have been characterized primarily by the pathologic anatomy of the gland, and more recently by the associated aberrations in iodine metabolism. With these methods of study, it has been difficult to differentiate factors influencing cell growth from those influencing hormone synthesis. Although cell growth and function parallel one another and are regulated chiefly by the thyrotropic hormone, there is some evidence of independent control.

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The purpose of this investigation was to procure another parameter of cell metabolism in the human thyroid, namely the incorporation of radiophosphorus. The total uptake of radiophosphorus reflects both cell growth and function. Cell growth is accompanied by the incorporation of phosphorus into organic protein compounds. Cell function is accompanied by the synthesis of organic iodine compounds and the transfer of energy through phosphate bonds.

In previous studies on the human thyroid, relationships between the total iodine and phosphorus contents have not been well established (2). They have revealed, however, that colloid contains phosphorus and that a high phosphorus content accompanies a scanty amount of colloid and an abundance of cells and nuclei (3, 4). In general, the phosphorus content of the thyroid varies with the degree of hypertrophy and the amount of epithelium (5). Recent investigations in the experimental animal have disclosed that the incorporation of labeled phosphorus into the thyroid is increased following thyrotropic hormone stimulation in the guinea pig, chicken and sheep (2, 6, 7) but not in the rat (8).

METHODS

Patients selected for study were those undergoing surgical treatment of diffuse toxic goiter, toxic nodular goiter, nodular goiter, or carcinoma of the thyroid. Control studies were made on a group of euthyroid patients undergoing operations unrelated to the thyroid.

Approximately twenty-four hours before operation, radiophosphorus[1] in a dosage of 3 microcuries per kilogram of body weight was administered intravenously. At the time of surgery, representative tissue specimens were procured from the thyroid gland and the adjacent sternocleidomastoid muscle, and a blood sample was withdrawn from a cervical or antecubital vein. The thyroid tissue was immediately sliced into sections measuring 2 to 3 mm. in thickness and a cross-sectional area of 1.5 to 2.0 cm. Triplicate specimens of thyroid and duplicate specimens of muscle were examined. The tissues were placed in weighed nickel plated steel planchets (1 inch in diameter), baked at 125°C. for twenty-four hours and weighed to 0.10 mg. Beta radioactivity per unit weight was appraised through an end-window Geiger-Mueller counter. Activity of thyroid and muscle was expressed as counts per minute per milligram and that of serum as counts per minute per 0.001 milliliter. In order to correlate the histologic picture with the P^{32} content, mirror specimens of the tissue assayed for P^{32} were submitted for pathologic examination. Those tissues exhibiting lymphoid infiltration or conspicuous degenerative changes on microscopic examination were excluded from the study.

Considerable variation was noted in the absolute values of P^{32} activity in the serum, thyroid, and muscle. In an effort to correct for differences in time of administration as well as serum levels of P^{32}, the ratio of counts per minute per milligram of thyroid to counts per minute per .001 milliliter of serum was employed as the most reliable index of P^{32} content and used for comparison of the normal, nodular, hyperthyroid and neoplastic glands.

[1] Procured from Abbott Laboratories, Oak Ridge, Tennessee, as sodium phosphate (P^{32}).

RESULTS

The results are shown in Table 1.

Euthyroidism (normal gland)

Control values were procured in 13 euthyroid subjects with diseases unrelated to disorders of the thyroid and in whom renal disease was not present. A number of these patients had parathyroid adenomas or had undergone exploratory operations because of the possibility of hyperparathyroidism. Their serum P^{32} levels were within the range of those seen in patients with nodular goiter, Graves' disease, and thyroid carcinoma.

The mean ratio of counts per minute per milligram of thyroid to counts per minute per .001 milliliter of serum was 23 (range, 15 to 31). Thyroid

TABLE 1. INCORPORATION OF P^{32} INTO TISSUES IN DISEASES OF THE THYROID GLAND

	Number of patients	Serum (c.p.m.*/ .001 ml.)	Thyroid (c.p.m./ mg.)	Muscle (c.p.m./ mg.)	Thyroid/ serum ratio
1. Euthyroidism (normal thyroid	13	.28† (.133)‡	6.1 (2.20)	7.1 (3.1096)	23 (5.93)
2. Graves' disease (treated with propylthiouracil or Tapazole and Lugol's sol.)	12	.35 (.226)	9.2 (3.52)	4.5 (1.57)	33 (15.8)
3. Graves' disease (treated with propylthiouracil or Tapazole)	7	.24 (.0458)	12.3 (7.30)	5.4 (.927)	49 (20.7)
4. Euthyroidism—nodular goiter (normal thyroid)	3	.37 (.0788)	8.2 (1.675)	8.3 (2.38)	22 (1.29)
5. Euthyroidism—nodular goiter (follicular adenoma)	12	.29 (.1321)	6.1 (3.60)	7.0 (3.292)	20 (9.22)
6. Euthyroidism—nodular goiter (fetal adenoma)	8	.37 (.1054)	14.8 (7.50)	6.9 (6.5)	40 (16.3)
7. Carcinoma	10	.30 (.0762)	21.8 (12.22)	7.8 (3.75)	71 (27.73)

* Counts per minute.
† Mean value.
‡ Standard deviation.

tissue was slightly less active than skeletal muscle. Cellular metabolism was appraised histologically on the basis of cell height, evidence of proteolytic activity at the margin of the colloid, and over-all cellular density (Fig. 1). Despite the variations in activity by histologic criteria, a relatively higher uptake could not always be documented in patients with an "active" gland compared to subjects with a gland which appeared to be quiescent.

Diffuse toxic goiter

Twenty patients aged 18 to 39 with diffuse toxic goiter were brought to a euthyroid state employing propylthiouracil or methimazole (Tapazole) with or without the addition of Lugol's solution. The average duration of treatment prior to surgery was four months.

Considerable variation in the uptake of P^{32} in serum, muscle and thyroid was observed. The mean thyroid-serum ratio in the patients treated with antithyroid drugs and Lugol's solution was 33 (normal, 23), with a range of

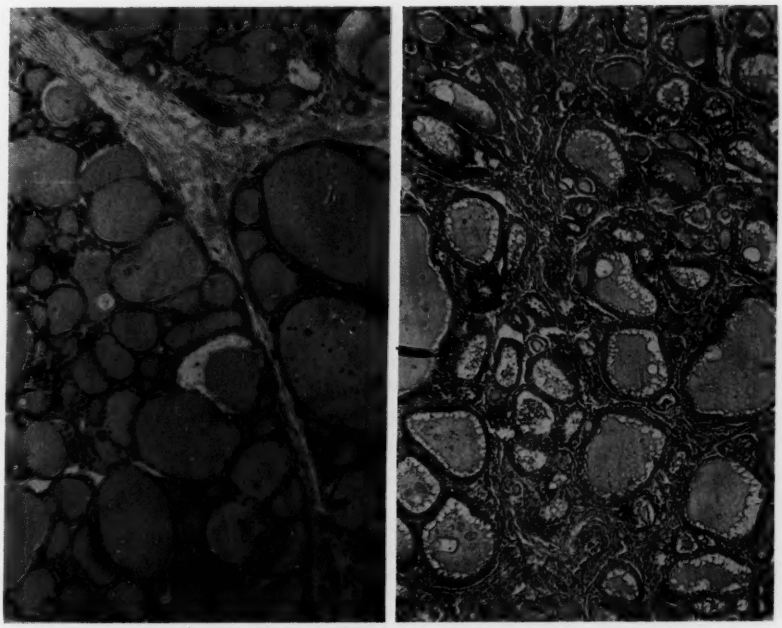

FIG. 1. A. Histologic features of the "normal" thyroid gland of a 38-year-old female operated upon for a parathyroid adenoma. The acinar epithelium appears relatively "inactive" with a P^{32} thyroid-serum ratio of 15.

B. Histologic characteristics of the "normal" thyroid gland of a 47-year-old male with a parathyroid adenoma. The P^{32} thyroid-serum ratio of 26 in this patient was slightly above the mean of normal (see Table 1). (H & E, 125×.)

6 to 70. Seven patients brought to a euthyroid state with propylthiouracil or methimazole alone had a mean thyroid-serum ratio of 49, with a range of 26 to 79.

Although the over-all activity was approximately 50 to 100 per cent greater than the mean for the normal thyroid, the increased P^{32} activity was most striking in a few patients with extreme hyperplasia in whom there was little or no colloid production (Figs. 2 and 3).

Of incidental interest was the finding of a decreased P^{32} uptake in muscle, thought to be the result of depression of all metabolic activity by propylthiouracil or methimazole (9) (Table 1).

Nodular goiter

The greatest variation in radioactivity was noted in the various types of nodular goiter. In attempting to relate P^{32} content to structure, results were grouped according to type of goiter, that is, follicular, macrofollicular, and microfollicular (fetal) adenomas. In general, the glands exhibiting the greatest cellularity had the highest P^{32} content. The mean thyroid-serum ratio for 8 fetal adenomas was 40, with a range of 8 to 55. In the "normal" thyroid tissue of patients with a fetal adenoma, the thyroid-serum ratio was 33. This relative increased activity in the normal-appearing gland could sometimes, but not always, be correlated with histologic evidence of increased functional activity. Evaluation of the normal "paranodular tissue" in other types of nodular goiter (that is, follicular and macrofollicular) seemed to show no essential difference over the control values for "normal" thyroid.

Of considerable interest was a patient with a multinodular goiter who had been taking 3 grains of desiccated thyroid daily for nine months, and 200 μg. of *l*-triiodothyronine daily for three months, and was then treated by thyroidectomy (Fig. 4). Despite the gross decrease in the size of the gland, there was no striking inactivity histologically, and approximately the same ratio of radiophosphorus incorporation was present in the adenomas as in the glands with normal TSH stimulation. This would sug-

→

FIGS. 2 & 3. Histologic characteristics of the thyroid in 5 patients operated upon for Graves' disease, showing varying degrees of epithelial hyperplasia and cell density. The P^{32} thyroid-serum ratios paralleled the microscopic appearance of "activity." (H & E, 120×.)

	T/S ratios
Fig. 2A	24.0
Fig. 2B	26.0
Fig. 3A	35.0
Fig. 3B	46.0
Fig. 3C	76.0

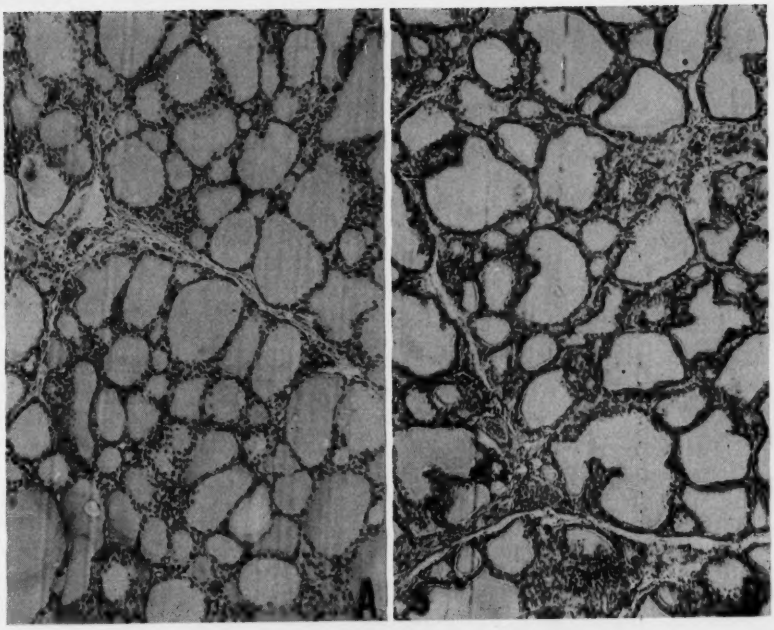

FIG. 2

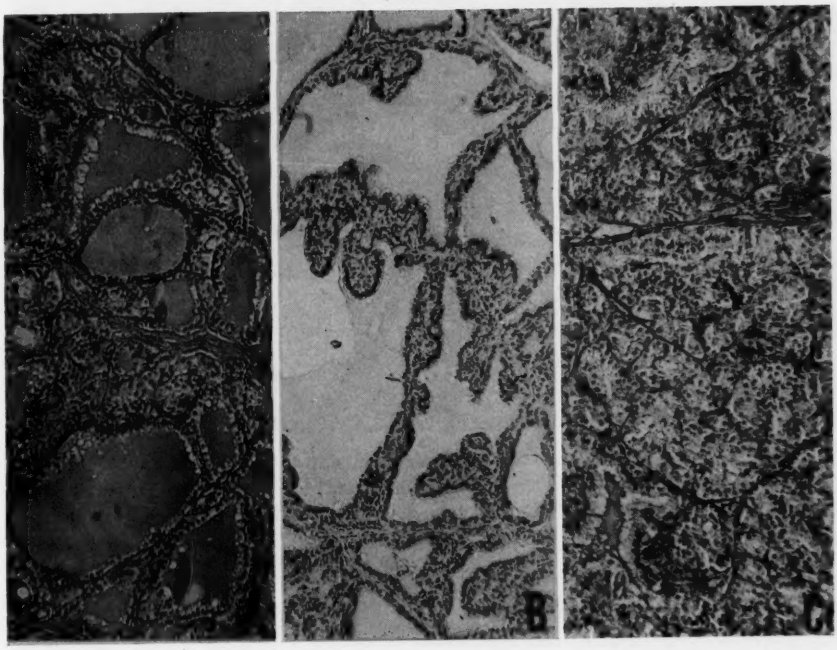

FIG. 3

gest that suppression of thyrotropic hormone plays a much greater role in depression of the functional activity of the gland than it does in growth. At the same time it may imply that these adenomas have already obtained some degree of autonomy and independence of TSH stimulation.

Carcinoma of the thyroid

Ten cases of thyroid cancer were studied. The P^{32} content was uniformly high (mean thyroid-serum ratio, 71) with few exceptions, and seemed closely correlated to the cellular density of the neoplasm (Figs. 5 and 6). In the patients with metastases, there was little basic difference between the total phosphorus content of the primary tumor and that of the metastasis. In a patient with an exceedingly well differentiated follicular and papillary carcinoma, no difference was detected between P^{32} uptakes in the primary tumor, normal thyroid, and a lymph-node metastasis. In this in-

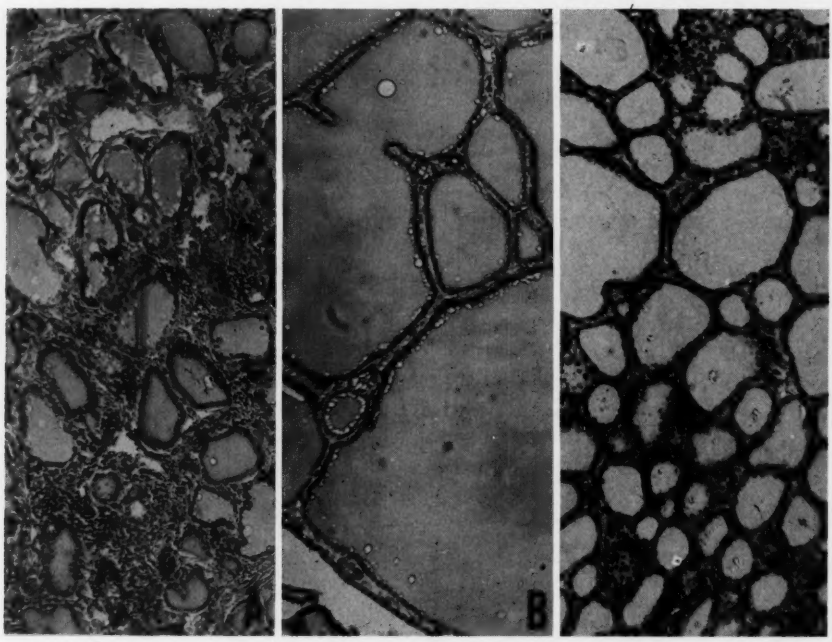

FIG. 4. Histologic appearance of a nodular goiter in a 34-year-old female who had been maintained on desiccated thyroid (3 gr. daily for nine months) and *l*-triiodothyronine (200 μg. daily for three months) preceding surgery. The P^{32} thyroid-serum ratios did not differ markedly from the mean values in "normal" patients. (H & E, 120×.)

T/S ratios

Fig. 4A (normal)	23.0
Fig. 4B (macrofollicular adenoma)	18.0
Fig. 4C (fetal adenoma)	46.0

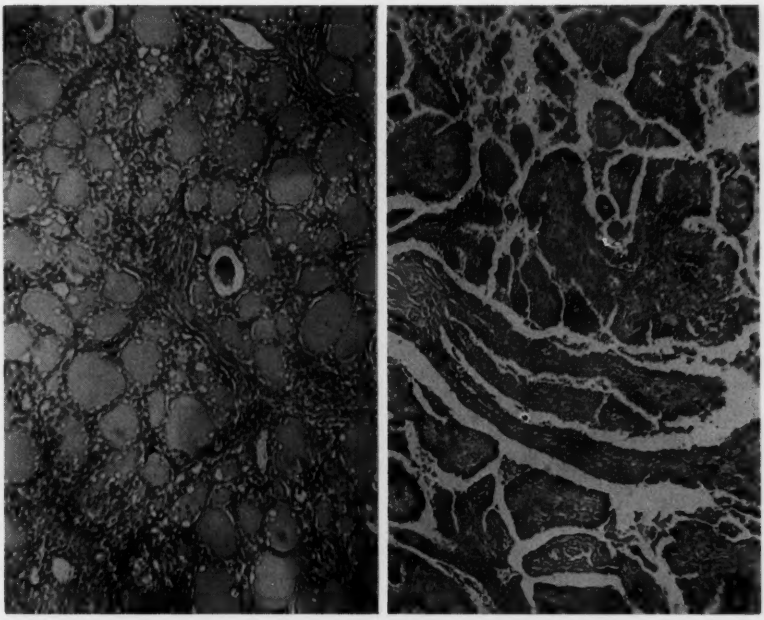

FIG. 5. Appearance of normal thyroid tissue, and of papillary adenocarcinoma in a 55-year-old male. (H & E, 120×.)

T/S ratios

Fig. 5A (normal)	28.0
Fig. 5B (papillary adenocarcinoma)	101.0

stance the cellular density and histologic appearance of the tumor and the metastasis were comparable to those of the normal thyroid.

A comparison of a fetal adenoma and carcinoma in another patient disclosed the higher P^{32} activity to be within the cancer (Fig. 6). In 5 patients treated for varying periods of time with *l*-triiodothyronine, values remained high with no suppression of the P^{32} thyroid-serum ratio of either the normal or the cancerous thyroid tissue.

DISCUSSION

Metabolism of the thyroid cell is related to: 1) *cell growth and division* as mediated through, but not entirely dependent upon thyrotropic hormone, and 2) *cell function* as mediated through the thyrotropic hormone level in conjunction with stored and available iodide, thyroid hormones and other agents modifying or blocking hormone synthesis. The response of the thyroid cell to thyrotropic hormone is apparently highly quantitative, in that the first response of an inactive or quiescent thyroid gland (in which there is still slight turnover of iodide depending upon available and stored hor-

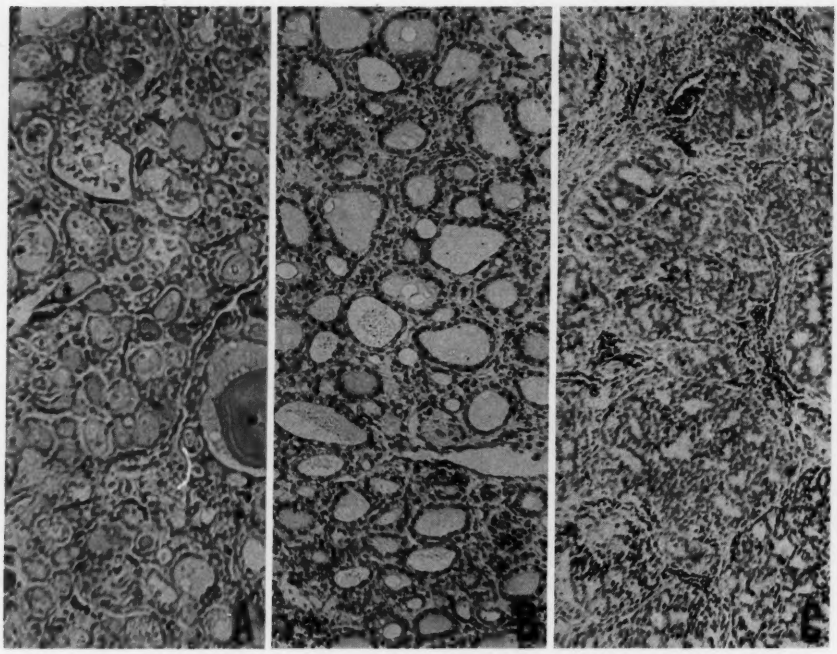

FIG. 6. Appearance of normal thyroid tissue, fetal adenoma, and cancer of the thyroid in a 55-year-old male. The P^{32} thyroid-serum ratios of P^{32} are seen to vary with the cellular density. (H & E, 120×.)

	T/S ratios
Fig. 6A (normal)	18.0
Fig. 6B (fetal adenoma)	42.0
Fig. 6C (adenocarcinoma)	112.0

mone) is increased functional activity (1, 10). This may occur before there is any pronounced change in thyroid weight, although there is histologic evidence of increased cell activity with increase in cell height and mitotic figures. At higher levels of stimulation, hyperplastic changes predominate and at this time the increased weight of the gland becomes a better index of its increased functional activity.

The total uptake of P^{32} in the thyroid would seem to be related primarily to: 1) incorporation into the nucleic acids of DNA and RNA, the former being related to deoxyribonucleoproteins of the cell nucleus whereas the RNA, located primarily in the endoplasmic reticulum (microsome fraction), is more related to the functional and enzymatic activity of the cell; 2) oxidative processes occurring within the cell which are accompanied by uptake of inorganic phosphates with formation of organic phosphates or

phosphate of the type of adenosinetriphosphate; and 3) incorporation into phospholipids, again reflecting metabolic activity of the cell.

Studies *in vivo* in the chick and guinea pig following stimulation with TSH have disclosed a pronounced increase in the labeled phosphorus content of the thyroid (2, 6). In the guinea pig, this could be correlated with increased cell height. A comparable increase in labeled phosphate in rat thyroid could not be demonstrated after TSH stimulation (8). In these experiments the administration of iodides, thiocyanates and the thiourea drugs had no effect on phosphorus content.

In vitro studies employing tissue slices of beef, sheep and human thyroid also demonstrated increased incorporation of P^{32} following stimulation with TSH (6, 11, 12). With the exception of cobaltous chloride, hormones and pharmacologic agents known to influence thyroid hormone synthesis (for example, iodides, thyroxine, propylthiouracil and potassium thiocyanate) had essentially no influence on the turnover of labeled phosphorus. In comparable studies on steer or heifer thyroids, TSH affected the synthesis of phospholipids only, and there was no effect upon the acid-soluble or protein phosphorus fractions. The alteration in phospholipid appeared to be fairly specific and was limited to thyroid cephalin and phosphoinositides.

Studies of thyroid metabolism in these surgical patients are complicated by changes incident to anesthesia, which may depress functional activity of the gland (13), as well as to the associated operative stress, which apparently increases thyroid hormone output (14–16). In addition, there is the problem of direct operative trauma and changes in blood supply. By waiting until the twenty-fourth hour after administration of P^{32}, it is probable that P^{32} levels have become fairly well stabilized and that minor changes in blood supply will no longer modify the end-results. In this study the data suggest that the amount of labeled phosphorus incorporated into the human thyroid at twenty-four hours reflects primarily cell density rather than functional (hormone synthesis) activity. Thus the highest content of labeled phosphorus was present in the neoplasm with a relatively high ratio of nucleus to cytoplasm and one in which hormone synthesis was probably minimal. In most glands there seemed to be fair correlation with secretory activity (employing histologic criteria of increased cell height and vacuolation of colloid) and an excellent correlation with cell density. The lack of alteration in P^{32} content in the human subject following administration of iodide, *l*-triiodothyronine, potassium perchlorate, and propylthiouracil is in keeping with results of tissue-slice studies, but is still somewhat surprising in view of the changes in the phospholipid fractions in the experimental animal.

It is concluded from these studies in man that total phosphorus content, as determined by radioactivity twenty-four hours after administration of

P^{32}, is related primarily to the density of the cell (probably reflecting nucleoprotein content) rather than the functional activity of the cell (hormone synthesis). The lack of suppression of P^{32} uptake, particularly by agents known to suppress stimulation by thyrotropin and formation of thyroid hormone, suggests an autonomy on the part of the cell that is somewhat greater than realized.

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THE EFFECT OF TRIIODOTHYRONINE AND THYROXINE UPON THE RATE OF RELEASE OF THYROID HORMONE IN VARIOUS THYROID STATES*

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ABSTRACT

The effect of triiodothyronine (T_3) or thyroxine (T_4) upon the thyroidal secretion rate was studied in 12 normal subjects, 15 hyperthyroid patients, 2 patients in the euthyroid stage of Graves' disease, and 1 acromegalic patient with hyperthyroidism. All normal subjects demonstrated prompt and nearly complete inhibition of thyroid hormone release following the administration of small doses of T_3 or T_4, whereas in all of the other subjects there was only a slight alteration in hormonal secretion rate in spite of massive doses of T_3 or T_4. One hyperthyroid patient showed normal suppression of thyroidal I^{131} uptake during administration of 75 micrograms of T_3 per day, but failure of suppression of the release rate when the dosage was increased up to 600 micrograms of T_3 per day. The mean release rate during the pre-T_3 control period was not significantly greater in the hyperthyroid group than in the normal group.

The findings are interpreted as favoring the hypothesis that hyperthyroidism is due to autonomous hyperactivity of either the thyroid or the pituitary gland, rather than simply a quantitative decrease in the sensitivity of the hypothalamo-pituitary apparatus to suppression by circulating thyroid hormones. Certain limitations in the method for measuring thyroidal secretion rate are discussed.

SEVERAL hypotheses have been advanced in the past regarding the etiology of Graves' disease. A plausible explanation for the disease must be compatible with the recent observation that the administration of thyroid hormone causes suppression of thyroidal radioiodide uptake in normal persons but regularly fails to do so in patients with Graves' disease (1–3). This highly consistent abnormality appears to be a fundamental trait, which invariably is present in the euthyroid stage of Graves'

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disease and which persists in many patients for several years after remission of hyperthyroidism has been induced by treatment (4–6).

Four hypotheses might conceivably fit with our current knowledge of the physiologic phenomena observed in hyperthyroidism: 1) the thyroid gland might be autonomously hyperactive regardless of the quantity of thyrotropin secreted by the pituitary; 2) the thyroid gland might still be under the control of pituitary thyrotropin but be hyper-responsive to normal or even to subnormal quantities of that hormone; 3) the suprathyroidal regulatory system acting through the pituitary might be functioning autonomously to secrete increased amounts of thyrotropin and not be capable of suppression by any attainable level of circulating thyroid hormone; and 4) the suprathyroidal system might secrete abnormally large amounts of thyrotropin but still be suppressible, albeit at a level of circulating thyroid hormone which is higher than that normally required for suppression of thyrotropin output.

The first concept has gained support from 3 recent reports of patients in whom hyperthyroidism developed in the face of apparent hypopituitarism (7–9). However, in each of these reported cases, there is no positive evidence that the pituitary was incapable of secreting thyrotropin at the time when hyperthyroidism appeared.

The second concept becomes very unlikely in view of the demonstration by Greer and Shull (10) that considerably larger quantities of thyrotropin are required to accelerate the thyroidal secretion rate in patients with Graves' disease than in normal subjects. Furthermore, this concept depends upon the unlikely assumption that the suppression of pituitary thyrotropin output by excessive amounts of thyroid hormone is never complete.

In considering the possible suprathyroidal mechanisms for the genesis of hyperthyroidism, a second observation by Greer and Shull (10) must be considered. In addition to the fact that thyrotoxic patients required approximately five to six times as much thyrotropin as euthyroid subjects before an increase in the release of thyroid hormone occurred, these workers showed in both types of subject a marked slowing of the secretion rate once the injections of thyrotropin were discontinued. These findings tended to favor the fourth hypothesis. The abnormally great resistance to exogenous thyrotropin would be compatible with the concept that the pituitary was already secreting an excessive amount of this hormone. Furthermore, the decrease in thyroidal secretion rate seen after discontinuing an effective dose of thyrotropin could have been due to inhibition of endogenous thyrotropin secretion by a further increase in the already elevated circulating thyroid hormone.

The present investigation was undertaken to test the fourth hypothesis

and thereby to allow a firmer choice between the two concepts of suprathyroidal disorder outlined here. Accordingly the effect of excesses of thyroid hormone on thyroidal secretion rate was studied.

MATERIALS AND METHODS

Twelve normal subjects, 15 hyperthyroid patients, 2 patients in the euthyroid stage of Graves' disease, and 1 acromegalic patient with hyperthyroidism were studied. All subjects were judged to be unequivocally either euthyroid or hyperthyroid by the usual clinical and laboratory criteria. All of the hyperthyroid patients had diffuse goiter.

Thyroidal secretion rate was determined, as previously described, by external scintillation counting over the thyroid gland following the administration of 25 to 300 microcuries (μc.) of I^{131} (11–13). Re-utilization of I^{131} liberated from the thyroid gland was prevented by the administration of 30 to 60 mg. of methimazole every six to eight hours after maximal uptake of radioiodide had occurred. Completeness of the block of reutilization was tested by 24-hour radioiodide uptake measurements performed at the end of the study, while the subjects were still receiving methimazole. In all but 2 (M. H. and F. A.) of the subjects tested, the 24-hour thyroidal I^{131} uptake was less than 5 per cent. The radioiodine uptake in Subject M.H. was 15 per cent when the I^{131} tracer capsule was given five hours after the dose of methimazole, whereas it was 3.5 per cent when the tracer was given two hours after the last dose of methimazole. In Subject F.A. the 24-hour I^{131} uptake was 40 per cent with 30 mg. of methimazole every eight hours.

The slope of the semilogarithmic curve described by serial counts over the neck represents the fractional rate of release of hormone from the thyroid gland (11–13). A standard I^{131} source was counted simultaneously to correct for physical decay and for variations in detector sensitivity. After the baseline slope was determined, varying doses of triiodothyronine (T_3) or thyroxine (T_4) were administered to all subjects. The straight line of best fit was determined graphically for each portion of the experiment and the daily fractional rate of release (λ) was calculated from the expression: $I/I^0 = e^{-\lambda t}$. Biologic half-life was calculated from the formula $T_{\frac{1}{2}} = 0.693/\lambda$. Statistical comparisons between different groups of patients were carried out by the *t*-test for unmatched data, and comparisons between different treatment periods were made by the *t*-test for paired data.

RESULTS

The data are summarized in Table 1. Eleven of the 12 normal subjects received 75–100 μg. of T_3 per day, either in fractional or single doses. All showed partial to complete inhibition of hormonal release, demonstrated by flattening of the release curve within twenty-four hours following the administration of the first dose. This effect on the slope of the release curve usually disappeared within twenty-four to forty-eight hours after the last dose of T_3 (Fig. 1). The mean percentage rate of release in the normal subjects during the pre-T_3 period was 6.81 ± 0.752 per cent per day compared to 1.05 ± 0.203 per cent per day during administration of T_3. This was a highly significant decrease ($t = 7.91$, $P < 0.001$). The mean release rate in the post-T_3 period was 5.52 ± 1.18 per cent per day, which was a significant rebound from the T_3 period ($t = 4.06$, $P < 0.005$). It should be noted that

TABLE 1. RATE OF RELEASE OF THYROID HORMONE BEFORE, DURING AND AFTER TREATMENT WITH THYROID HORMONES

Patient		Maximum dose (μg. per day)		Fractional daily rate of I^{131} release				
				1	2	3	Differences	
Name	Age & Sex	T_3	T_4	Tapazole	Tapazole plus T_3 or T_4	Tapazole	1−2	3−2
Normal								
J.S.	63 F	75		.0392	.0175	.0228	.0217	+.0053
P.H.	69 F	75		.0724	.0200	.0745	.0524	+.0545
D.S.	50 F	100		.1050	.0151	.0888	.0899	+.0737
L.K.	43 M	75		.0481	.0094	.0441	.0387	+.0347
M.K.	62 F	75		.1100	.0095	.1342	.1005	+.1247
H.H.	59 M	100		.0428	.0097	—	.0331	—
L.Q.	37 M	75		.0866	.0200	—	.0666	—
S.C.	29 F	75		.0865	.0000	.0693	.0865	+.0693
C.T.	48 M	100		.0666	.0048	.0166	.0618	+.0118
J.H.	28 F	75		.0459	.0058	.0233	.0401	+.0175
A.N.	64 F	75		.0343	.0000	.0248	.0343	+.0248
F.B.	48 F		6000	.0797	.0143	.0533	.0654	+.0390
Mean				.0681	.0105	.0552	.0576	+.0455
Standard Error				± .00752	± .00203	± .0118	± .00728	± .0112
Hyperthyroid								
A.W.	37 F		6000	.1865	.1865	.1865	0.0	0.0
L.R.	56 M	500		.0679	.0582	.0368	.0097	−.0214
F.A.	47 M	1200		.0619	.0281	.0191	.0338	−.0090
M.S.	42 F	1600		.0866	.0678	—	.0188	—
M.St.	63 F	75		.0686	.0230	.0230	.0456	0.0
B.S.	26 F	600		.1980	.1612	—	.0368	—
E.C.	54 F	300		.0680	.0680	—	0.0	—
J.M.	25 F	600		.0625	.0541	—	.0084	—
V.U.	51 F	600		.0545	.0365	—	.0180	—
M.Ma	43 F	150		.1034	.0950	.0950	.0084	0.0
M.H.	52 F	375		.0408	.0408	.0241	0.0	−.0167
M.T.	72 F	150		.0450	.0317	.0317	.0133	0.0
H.O.	66 F	75		.0607	.0342	.0206	.0265	−.0135
E.S.	64 F	500		.0537	.0537	.0280	0.0	−.0257
M.M.	26 F	600		.0800	.0499	.0268	.0301	−.0231
Mean				.0825	.0659	.0492	.0166	−.0118
Standard Error				± .0127	± .0123	± .0168	± .00386	± .00361
Euthyroid stage of Graves' disease								
L.W.	63 F	600		.0987	.0737	—	.0250	—
Z.H.	61 F	400		.1692	.1195	—	.0497	—
Acromegalic patient with hyperthyroidism								
L.B.	37 M	2000		.0815	.0665	—	.0150	—

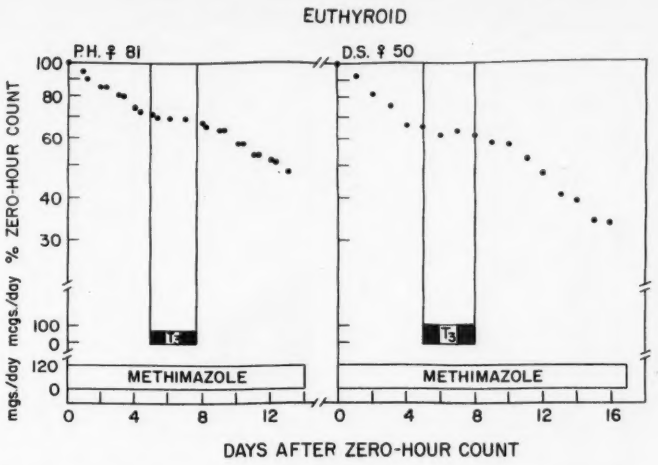

FIG. 1. Thyroidal secretion rate curves in 2 normal subjects receiving T_3. In this and all subsequent figures the radioactivity, after correction for physical decay, is plotted semi-logarithmically against time. T_3 caused a prompt decrease in secretion rate in both subjects.

the slope in the post-T_3 period was usually less than in the pre-T_3 period, although the mean difference was not significant ($t=2.22$, $P>.05$).

The twelfth normal subject, who received 6 mg. of thyroxine over a 24-hour period, demonstrated immediate and sustained flattening of the release curve for fourteen days. The slope gradually returned to its baseline level thirty days after administration of thyroxine. Both the serum protein-bound iodine level and the basal metabolic rate became significantly elevated (Fig. 2).

In contrast to the normal subjects, the 14 hyperthyroid patients showed little or no change in thyroidal secretion rate when T_3 was administered in doses up to 1600 μg. per day. Definite elevation of the basal metabolic rate was usually produced by the quantities of T_3 administered (Fig. 3). The mean percentage release rate in the hyperthyroid patients during the pre-T_3 period was 8.25 ± 1.27 per cent per day and fell slightly to 6.59 ± 1.23 per cent per day during administration of T_3. This difference was significant because of its consistency ($t=4.3$, $P<0.001$). However, the slope continued to fall in the post-T_3 period; the mean release rate reached 4.92 ± 1.68 per cent per day, a further significant fall from the T_3 level ($t=3.27$, $P<0.01$, based on the 10 cases with post-T_3 data). Thus the post-T_3 control period showed a far lower release rate than the pre-T_3 control period ($t=4.94$, $P<0.001$, based on the same 10 cases)—an expression of the gradual bowing of the release curve which was seen frequently but ap-

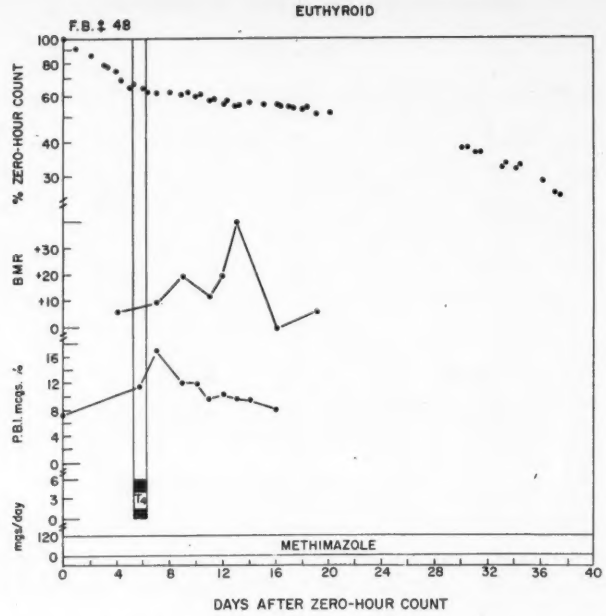

FIG. 2. The effect of 6 mg. of T_4 on serum PBI level, BMR, and thyroidal secretion rate in a normal subject. The decrease in secretion rate outlasted the effects on BMR and PBI.

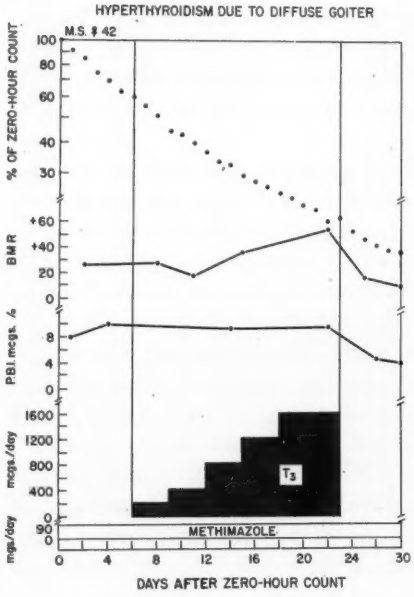

FIG. 3. Lack of effect of large doses of T_3 on the slope of the thyroidal secretion curve in a hyperthyroid patient. There was a significant increase in the BMR.

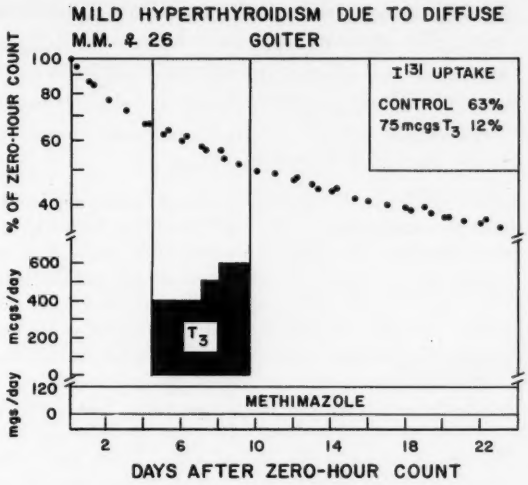

FIG. 4. Lack of effect of T_3 on the thyroidal secretion rate in a patient in whom small doses of T_3 produced normal suppression of the thyroidal I^{131} uptake. This figure also illustrates a marked degree of the upward concavity of the release curve which was seen in many of the hyperthyroid subjects, but which was not related in time to the administration of T_3.

peared to be unrelated to the administration of hormone (*e.g.*, Fig. 4). An additional hyperthyroid patient who was given 6 mg. of thyroxine over a 24-hour period also failed to show any alteration in the rate of hormonal release, although the basal metabolic rate and serum protein-bound iodine level were increased (Fig. 5).

Certain comparisons may be made between the normal and the hyperthyroid groups. First of all, there was no significant difference between the release rates of the two groups in either the pre-T_3 or the post-T_3 control periods ($t=0.94$ and 0.29 respectively). The mean fall in release rate while receiving T_3 or T_4 was only 1.66 ± 0.386 per cent per day in the hyperthyroid patients, but the mean fall in the normal subjects was significantly greater, namely, 5.76 ± 0.728 per cent per day ($t=5.25$, $P<0.001$). When the T_3 period was compared with the post-T_3 period, the difference between the two groups of subjects was significant, not only quantitatively but qualitatively. All of the normal subjects showed an increase in release rate after T_3 was withdrawn, whereas all of the hyperthyroid patients showed either a decrease in release rate or no change. The mean post-T_3-minus-T_3 difference for the normal subjects was 4.55 ± 1.12 per cent per day, whereas that for the hyperthyroid patients was -1.18 ± 0.361 per cent per day ($t=4.86$, $P<0.001$).

One patient, M.M., with mild hyperthyroidism due to diffuse goiter was of unusual interest. She showed the same failure of suppression of hormonal release rate as all the other hyperthyroid patients in this study, despite doses of T_3 up to 600 μg. per day. However, she demonstrated normal suppression of radioiodide uptake (from 63 per cent down to 12 per cent) with a dosage of only 75 μg. of T_3 per day (Fig. 4).

M.M. was a 26-year-old Negress who was first seen twenty-two months prior to the study, at which time she was judged to be unequivocally hyperthyroid by the usual clinical and laboratory criteria. The serum protein-bound iodine level was 10.9 μg. per 100 ml. and the 6-hour and 24-hour thyroidal radioiodide uptake was 56 and 67 per cent, respectively. She was treated with propylthiouracil, which she discontinued after only three months. She was next seen at the time of this study. She reported that she had remained asymptomatic for approximately one year after discontinuing the medication and had then noted recurrence of nervousness, fatigue, sweating, weight loss, and palpitation during the seven months preceding this study. On examination her height was 5 feet 6 inches, and weight 102 pounds. The pulse was 110 and the blood pressure 135/60 mm. Hg. The skin was warm, moist and silky. There was a fine tremor of the extended fingers. Stare and lidlag were present but there was no evidence of infiltrative exophthalmos. The thyroid gland was diffusely enlarged (estimated weight 70 grams). The concentration of serum protein-bound iodine was 9.8 μg. per 100 ml. and the thyroidal 24-hour radioiodide uptake was 63 per cent.

The described studies were carried out and the patient was treated with methimazole, which she again discontinued voluntarily after six months. Three months later, mild symptoms recurred, and after another three months she presented to the clinic with symptoms and signs of mild hyperthyroidism for the third time. The serum protein-bound iodine level was now 8.5 μg. per 100 ml. The thyroidal radioiodide uptake was 45 per cent in twenty-four hours, and again it was suppressed (falling to 16 per cent) after eight days of administration of T_3 in a dosage of 75 μg. per day.

Two patients in the euthyroid stage of Graves' disease were studied. They had at all times been clinically euthyroid but suffered from infiltrative exophthalmos. The serum protein-bound iodine level and thyroidal radioiodide uptake were normal. Doses of T_3 up to 600 μg. per day produced no change in the release rate in either patient (Fig. 6).

Similarly, the release curve in a 37-year-old male with acromegaly and hyperthyroidism was not altered following the administration of 2000 μg. of T_3 per day (Fig. 7).

DISCUSSION

The phenomenon of failure of inhibition of thyroid function by the administration of thyroid hormone in Graves' disease, previously demonstrated by radioiodide uptake studies, has been extended in the present study to include the hormone-release function. All of the 12 normal subjects showed prompt and significant suppression of the thyroidal secretion rate following the administration of T_3 or T_4. On the other hand, only a very slight alteration in the rate of hormonal release occurred, in spite of

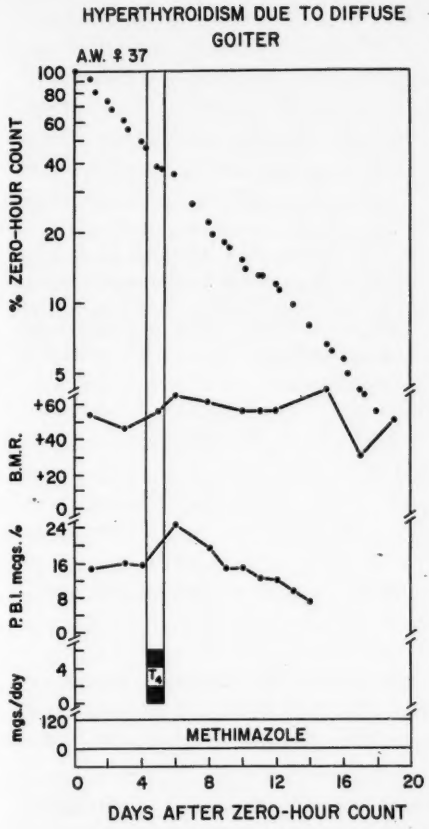

Fig. 5. The effect of 6 mg. of thyroxine on the serum PBI level, BMR, and thyroidal secretion rate in a patient with severe hyperthyroidism. Contrast with Figure 2.

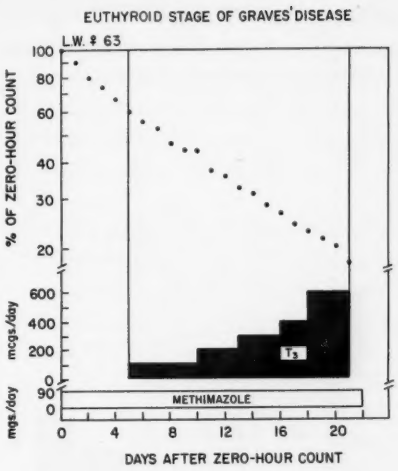

Fig. 6. The lack of effect of T_3 on the thyroidal secretion rate of a patient in the euthyroid stage of Graves' disease.

massive doses of T_3 or T_4, in 15 hyperthyroid patients, 2 patients in the euthyroid stage of Graves' disease, and 1 acromegalic patient with hyperthyroidism.

Although a slight decrease in the rate of hormone release in some hyperthyroid patients appeared to coincide with the administration of T_3, a study of the time factors in individual curves indicated that there was no cause-and-effect relationship. Furthermore, analysis of all of the data indicated that there was a progressive decrease in the slope of the release curve with increasing duration of methimazole therapy. This was confirmed by the significantly lower release rate in the post-T_3 period as compared to that in the pre-T_3 period. It is of interest that the euthyroid subjects who showed a marked reduction in the release rate during administration of T_3 invariably exhibited a sharp rebound to a steep slope during the post-T_3 period. In contrast, the release curves for the hyperthyroid patients merely

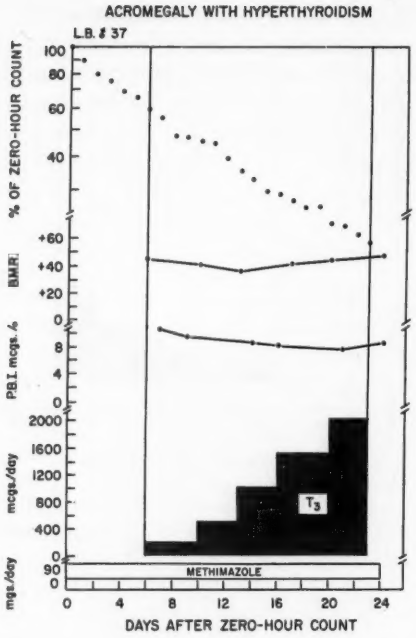

FIG. 7. The lack of effect of massive doses of T_3 on the thyroidal secretion rate of an acromegalic patient with hyperthyroidism. In this instance, the BMR was also not affected.

decreased further in slope during the post-T_3 period. For these reasons, it appeared safe to conclude that the slight observed alteration in slope could not be attributed to the administration of thyroid hormones in the hyperthyroid patients.

Very large doses of thyroid hormone were employed in the hyperthyroid patients in order to determine whether the suprathyroidal mechanism was at all suppressible in Graves' disease. The presumption was that larger amounts of hormone would be needed in hyperthyroid than in normal persons in order to force the circulating level of hormone to a point above the setting of the "thermostat." Hence, doses up to 2000 μg. per day of T_3 were given, and an elevation of the basal metabolic rate and some increase in the patients' symptoms usually resulted. The significance of the doses of thyroid hormone used in this study becomes clearer by comparison with estimates of the rate of hormone synthesis and degradation in hyperthyroidism. By the I^{131}-turnover method of Berson and Yalow, the daily secretion rate was estimated at from 420 to 925 μg. of hormonal iodine per day in 9 hyperthyroid patients (14). Ingbar and Freinkel, utilizing I^{131}-labeled thyroxine, indicated a range of 530 to 1101 μg. per day for the same parameter (15). By contrast, reasoning from equivalent replacement doses in the treatment of myxedema, the 6 mg. of T_4 administered in this study would

be equivalent to a secretion rate of 2100 μg. of hormonal iodine, and 2000 μg. of T_3 would be metabolically equivalent to a secretion rate of 1900 μg. of hormonal iodine per day. Hence, the doses used may be assumed to have far more than doubled the amount of thyroid hormone available to act on the hypothalamo-pituitary mechanism, even assuming that the degradation of the exogenous hormone may have been somewhat more rapid than at the patients' endogenous level.

Triiodothyronine was administered to most patients, rather than thyroxine or desiccated thyroid, in order to obviate the administration of compounds containing amounts of iodine which might in themselves inhibit thyroid secretion. The patients given thyroxine, however, did not exhibit a response different from those receiving triiodothyronine.

The failure of very large doses of T_3 or T_4 to produce suppression of the thyroidal secretion rate in hyperthyroid patients strongly favors the concept that either the thyroid gland or the suprathyroidal regulatory system is functioning autonomously and that the disorder of regulation is qualitative rather than simply a quantitative abnormality. These results run counter to the working hypothesis presented in the Introduction and leave unexplained the decrease in secretion rate previously reported to follow administration of thyrotropin and its withdrawal in hyperthyroid patients. It is possible that this decrease might be due to the inability of the thyroidal enzyme system involved in the secretion of hormone to keep functioning optimally when the stimulus of excessive exogenous thyrotropin is suddenly withdrawn.

The "bowing" of the release curves discussed previously is itself of some interest. It was not seen frequently in previous studies (12, 13) and its appearance in the present study is unexplained. One interpretation is that the fractional rate of hormone release actually decreases as the store of hormone in the thyroid gland falls to low levels. It has already been established that the absolute amount of hormone secreted declines in proportion to the declining hormone stores (13, 16). The present finding suggests that the regulation of release may become even more restrictive under some circumstances. Another explanation might be simply that blocking of reutilization is incomplete due to fluctuation in the effective level of methimazole. Escape from antithyroid drug action a few hours after administration of large single doses has been demonstrated by Stanley and Astwood (17) and by Berson and Yalow (18), and was confirmed under the conditions of the present study. If the thyroid gland becomes increasingly more avid for available iodide as methimazole treatment continues, as seems likely, a progressively greater fraction of the I^{131} released by the thyroid and metabolized by the tissues would be trapped by the gland. If then the drug-induced block on organic binding were intermittent, the gland would

re-incorporate an increasing fraction of available I^{131}, resulting in the upward concavity of the apparent release curve seen in this study. A third possibility is that some patients were gradually entering a state of remission of the disease.

It is of interest that, during the control period, no significant difference existed between the release rates in the euthyroid and hyperthyroid patients in this study. In a previous study, one of the authors presented data which indicated a consistently greater release rate in hyperthyroid than in euthyroid subjects, with very little overlap between the two groups (13). The lack of a significant difference may reflect wide variations among hyperthyroid patients in the amount of hormone stored in the thyroid gland. Earlier studies have long indicated that thyroidal hormone stores were subnormal in hyperthyroidism, but Berson and Yalow found greatly enlarged thyroidal hormone pools in some hyperthyroid patients (14). A large hormone pool could lead to a "normal" fractional rate of I^{131} release, even though decidedly supernormal *amounts* of hormone were being released per unit time. Whatever the explanation, it should be emphasized that the slope of the methimazole-blocked I^{131} release curve cannot be used as a diagnostic test for the state of thyroidal function.

The present study afforded an opportunity to compare the effect of thyroid medication on the thyroidal uptake of I^{131} with its effect on the hormone release function. In general, the results by the two methods were quite comparable. However, the one exception is of considerable interest. A patient with mild but definite hyperthyroidism showed ready suppression of I^{131} uptake when T_3 was administered, but no suppression of the release rate. These findings, if observed in subsequent studies, would suggest that the regulation of the I^{131} uptake function in hyperthyroid patients may on occasion be qualitatively different from the regulation of release of hormone.

Acknowledgment

The technical assistance of Ethel Plummer is gratefully acknowledged.

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ASSAY OF THYROID FUNCTION IN DIABETES MELLITUS

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ABSTRACT

Thyroid function was assayed in 25 unselected patients with diabetes mellitus by measuring thyroidal and renal clearance rates of radioiodine (I^{131}). The thyroidal clearance rate of radioiodine was within the normal range in all patients; the renal clearance rate was markedly decreased in patients with intercapillary glomerulosclerosis but was within normal limits in the other diabetic subjects, including 2 with retinopathy without nephropathy. This indicates that thyroidal handling of iodine is normal in all phases of diabetes mellitus and that altered urinary excretion patterns are a reflection of the renal lesion rather than of the underlying systemic disease.

PATIENTS with myxedema or the nephrotic syndrome often present with a puffy appearance, edema, anemia, lethargy, hypercholesterolemia and a low concentration of serum protein-bound iodine. Despite these common clinical characteristics, malfunction of the thyroid gland is not demonstrable in nephrosis, no matter what the etiology (1–3). Perry and Hughes (4) showed that the diabetic patient with the nephrotic syndrome due to intercapillary glomerulosclerosis excretes radioactive iodine (I^{131}) more slowly than does the normal subject, sharing this defect with other patients who have advanced renal disease. Because the pathologic spectrum of diabetes mellitus is a broad one, it seemed pertinent to evaluate thyroid function throughout.

METHODS OF STUDY

Thyroid function was assayed in 25 unselected diabetic patients (Table 1). The method of Oddie *et al.* (5-7) was used to calculate K_1 (the thyroidal iodide clearance rate factor) and K_2 (the renal iodide clearance rate factor). K_1 refers to the fraction of the total iodide space cleared of iodide per minute by the thyroid gland, and K_2 indicates the fraction of the total iodide space cleared by the kidneys per minute. Normal values

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for K_1 are 0.23×10^{-3} minute^{-1} to 3.85×10^{-3} minute^{-1} (median 0.55); and for K_2, 0.4–3.9×10^{-3} minute^{-1} (median 1.3). The normal range for 24-hour thyroidal I^{131} uptake in this laboratory is 8–62 per cent. The serum protein-bound iodine level was determined by the method of Barker (8); normal values in our laboratory are 3.5–7.5 μg. per 100 ml.

TABLE 1. DATA ON THYROIDAL AND RENAL FUNCTION IN 25 PATIENTS WITH DIABETES MELLITUS

Patient		Duration of diabetes (yrs.)	Blood levels		Clearance rate factors		Thyroidal 24-hr. I^{131} uptake
Case No.	Sex & Age		BUN (mg./100 ml.)	PBI (μg./100 ml.)	K_1 (thyroidal)	K_2 (renal)	(%)
1	M 63	$\frac{6}{12}$	22		0.44	1.01	
2	F 62	27	7		0.75		
3	F 79	new	18	6.3	0.58	1.30	22.1
4	F 63	$\frac{2}{12}$	22		0.81	1.07	28.2
5	M 60	3	20	4.7	0.49	0.92	
6	F 73	2		5.1	1.66	3.74	34.3
7	F 62	4	12		1.49	2.14	
8	F 38	3	14		0.93	3.38	18.4
9	M 76	new	16		1.10	0.85	38.9
10	F 57	6	23	4.7	0.51	2.09	22.1
11	F 56	$1\frac{7}{12}$	20	7.5	0.25	1.67	
12	M 67	2	17	7.5	0.57	1.86	
13	F 65	new			0.54	2.09	21.5
14	F 68	2			2.00	0.65	
15	F 55	new	19	6.5	0.52	2.35	
16	F 50	new	15	5.6	1.31	1.75	
17	F 64	6		5.6	1.00	2.24	
18	F 42	7	22	5.1	0.4	1.9	
19	M 59	new	20	5.6	0.71	2.6	
20	M 53	new	14		1.16		
		Retinopathy without nephropathy					
21	M 36	8		4.9	0.95	2.05	
22	M 56	25			1.27	0.93	36.4
		Nephropathy					
23	M 42	15	59	3.7	0.77	0.23	46.0
24	M 28	23	126	3.3	0.82	0.10	38.9
25	M 33	13	81		0.63	0.17	48.9
Range					0.25–2.0	0.10–3.74	
Median					0.77	1.75	

RESULTS

The thyroidal and renal clearance rate factors and 24-hour thyroidal I^{131} uptakes are shown in Table 1. Patients with diabetes mellitus but without evidence of renal complications had normal values, whereas those with glomerulosclerosis had normal thyroidal rates, low renal rates and high-normal 24-hour I^{131} uptakes. The concentration of serum protein-bound iodine was within the normal range in 11 patients without renal disease; in 2 patients with renal involvement, it was 3.3 and 3.7 μg. per 100 ml., respectively.

COMMENT

This study indicates that thyroid function as measured by I^{131} clearance is normal in diabetes mellitus and that the abnormal renal rate observed in intercapillary glomerulosclerosis cannot be attributed to diabetes mellitus. That this is so should not be surprising, because of the nature of the known changes in renal function in diabetes. Corcoran *et al.* (9) demonstrated depression of the glomerular filtration rate in 5 such patients, and Bricker and Hlad (10) presented evidence that renal iodide clearance parallels, though it is always less than, inulin clearance over a wide range of values for both normal and sick patients. Under such conditions an increased 24-hour uptake of I^{131} by the thyroid might be expected, because decreased renal clearance would result in increased time for the circulation of iodine through the gland.

Diabetic retinopathy, which may be regarded as a harbinger of diabetic nephropathy, was seen twice in this series in patients without gross evidence of renal disease. In both instances, I^{131} clearance rate factors were normal.

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CHANGES IN PLASMA LEVELS OF 17-HYDROXY-CORTICOSTEROIDS DURING THE INTRAVENOUS ADMINISTRATION OF ADRENOCORTICOTROPIN (ACTH).

IV. RESPONSE TO PROLONGED INFUSIONS OF SMALL AMOUNTS OF ACTH*

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ABSTRACT

Continuous intravenous infusions of ACTH at rates of 0.8 to 1.4 I.U. per twenty-four hours maintained the plasma concentration of free 17-hydroxycorticosteroids above the maximal levels attained spontaneously during a diurnal variation. No diurnal variation of the plasma level of free 17-hydroxycorticosteroids was observed during these sustained elevations produced by infusions of ACTH at rates greater than 0.8 I.U. per day. In males the maximal plasma 17-hydroxycorticosteroid levels can probably be achieved during the normal diurnal variation by the secretion of ACTH at a rate of less than 4 I.U. per day for a period of less than four hours. Continuous infusion of very small amounts of ACTH increased the capacity of the adrenal to produce 17-hydroxycorticosteroids, whether or not increased steroid secretion resulted from the initial infusion.

THE response of plasma free[1] 17-hydroxycorticosteroids to the intravenous infusion of large amounts of adrenocorticotropin (ACTH) over short periods of time has been studied by many investigators and has become established as a useful means for testing adrenocortical capacity to produce hydrocortisone (1, 2). This investigation was undertaken to study the plasma 17-hydroxycorticosteroid response to small doses of ACTH given intravenously over prolonged periods of time.

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[1] The term "free" refers to the methylene chloride-soluble (nonconjugated) compounds rather than the presence or absence of binding to protein in plasma.

METHODS

Commercial preparations of ACTH[2] were dissolved in physiologic sodium chloride solution, and 1 mg. of heparin, 10 γ of dihydrostreptomycin and 10 γ of penicillin were added per ml. of solution. Fresh solutions were made up each day and stored in a refrigerator at 40° C. An American Instrument Company motor-driven compensator with a 20-ml. syringe was used to deliver the solution at a constant rate of 2 ml. per hour. The syringe was refilled every eight hours. Plastic tubing connected the syringe to a 20-gauge needle, which was placed in a vein in the subject's forearm. Blood specimens were drawn from an 18-gauge, Cournand-type needle inserted in a vein in the subject's other forearm. The needles were placed so that they did not interfere with movement at the wrist and elbow. After each specimen of blood was drawn, 3 mg. of heparin in 0.3 ml. of physiologic salt solution was injected into the needle before the trocar was replaced. Before the syringe was attached to obtain a blood specimen, a tourniquet was tightened on the subject's arm and a 1 to 2-ml. portion of blood was allowed to wash out the needle. A volume of 5 to 15 ml. of blood was drawn for each specimen, and mixed with 1 mg. of heparin. The plasma was separated by centrifugation within one hour and stored in a deep-freeze at −30° C. to await analysis. The methods used in this investigation for the determination of plasma steroids have been described by Eik-Nes (3).

During the study, the subjects were kept in bed or allowed to sit in a chair. The infusion pump was placed on a cart. The pump was in constant operation with the exception of 30-second periods when the subjects walked beside the infusion pump between transfers of the electrical plug-in connection from bedroom to bathroom and vice versa. The subjects' meals and the night-time sleeping patterns were little altered by the experiment, although it was necessary to awaken them to obtain the night-time plasma specimens.

RESULTS

Subject A (weight 98 pounds, height 63 inches)

The patient was a 52-year-old white male with adrenal insufficiency secondary to inadequate production of ACTH. (A detailed report will be made later (4).) He was emaciated due to an esophageal carcinoma with metastases. A gastrostomy tube was in place. He had been maintained on 20 mg. of hydrocortisone, orally, each day. Beginning twenty-two days before this study, he was given 10 I.U. of ACTH intramuscularly every six hours, and after five days the hydrocortisone was discontinued. The last intramuscular injection of ACTH was given six hours before the study began.

The results are shown in Figure 1. There was a sharp rise in the level of plasma 17-hydroxycorticosteroids on Day 1 with a daily dose of 5.8 I.U. of ACTH. On Days 2 and 3, the dose was reduced to 2.8 and 1.4 I.U., but the concentration of plasma steroids continued to rise, reaching a level between 37 and 42 γ per 100 ml. of plasma. This concentration is considerably greater than the values observed in normal subjects during the usual di-

[2] ACTH was kindly supplied for this study by the Upjohn Company, Kalamazoo, Michigan, and Armour Laboratories, Chicago, Illinois.

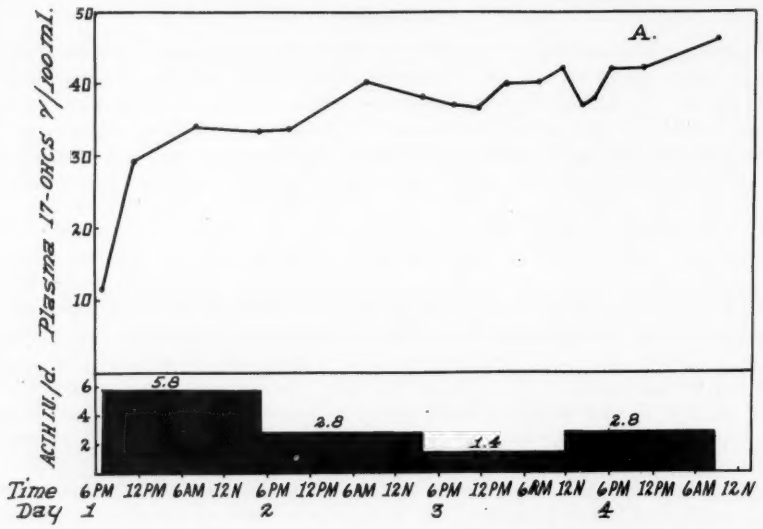

FIG. 1. *Subject A*. The hours and the days of the study are plotted along the abscissa. The rate of infusion of ACTH and the concentration of plasma 17-hydroxycorticosteroids are indicated along the lower and the upper part of the ordinate, respectively.

urnal variation (5). On Day 4, with a daily dose of 2.8 I.U., the average plasma steroid level was higher than on the preceding days.

There were small fluctuations in plasma steroid concentration after the middle of Day 2. However, these minor changes did not have the pattern of the normal diurnal variation (5).

Subject B (weight 160 pounds, height 70 inches)

The patient was a 52-year-old male who had been admitted to the hospital with pneumonococcal pneumonia. He was studied nineteen days after admission, at a time when he was clinically well. The results are shown in Figure 2. While receiving an infusion of ACTH at a rate of 1.2 I.U. per day, this subject maintained, for a brief period, a plasma 17-hydroxycorticosteroid concentration of 28 γ per 100 ml., a level above the maximum achieved by normal subjects at any time in the day. The study was ended when the ACTH solution infiltrated into the subcutaneous tissues late in the first day.

Subject C (weight 147 pounds, height 70 inches)

The subject was a healthy, 30-year-old, white male volunteer. During administration of 2.9 I.U. of ACTH per day there was a striking rise in

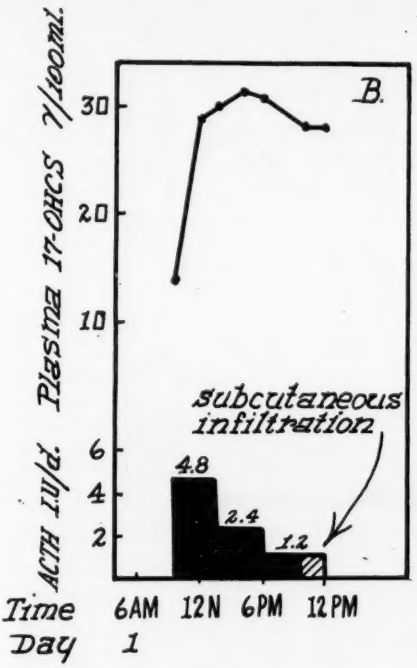

FIG. 2. Study on *Subject B.*

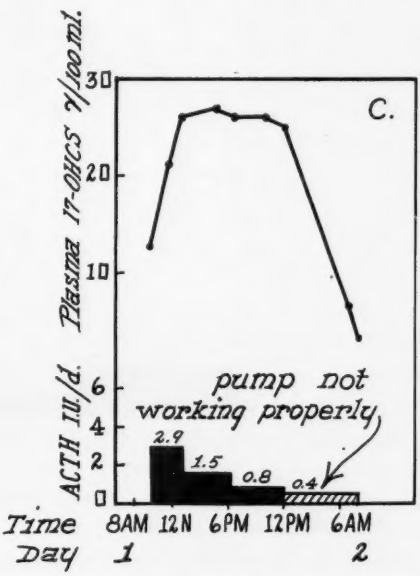

FIG. 3. Study on *Subject C.*

plasma 17-hydroxycorticosteroids during the first four hours to a concentration of 26γ per 100 ml. (Fig. 3). The plasma steroids remained at about this level for two subsequent 5½-hour periods during administration of 1.5 and 0.8 I.U. of ACTH daily. Due to an error in adjusting the pump, little ACTH was infused after midnight on the first day and the experiment was ended.

Subject D (weight 162 pounds, height 70 inches)

This subject was a 28-year-old, white male volunteer. During Day 1, with a dosage of 3.9 I.U. of ACTH daily, there was a rise in the level of plasma 17-hydroxycorticosteroids to 42 γ per 100 ml. (Fig. 4). The rise in the first six hours was smaller than the average rise in normal subjects (Fig. 5) receiving 25 I.U. of ACTH over a six-hour period (1); however, the value at the end of Day 1 was as high as that usually produced during twenty-four hours of continuous infusion of large doses of ACTH (1). The concentration rose to a level around 48 γ per 100 ml. during Day 2, with an ACTH dosage of 1.9 I.U. per day. On Day 3, with a dosage of 1.0 I.U. of ACTH

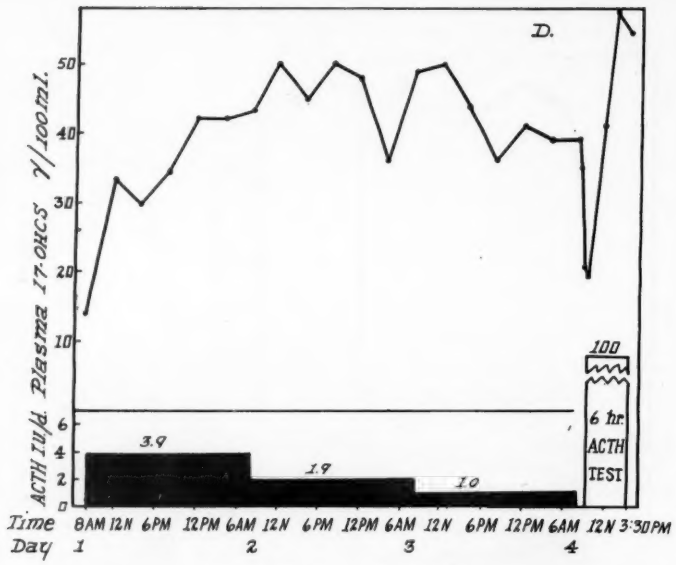

FIG. 4. Study on *Subject D.*

daily, the plasma 17-hydroxycorticosteroid level fell to about 39 γ per 100 ml.

In the hour beginning thirty minutes after the completion of the constant infusion on Day 4, there was a rapid fall of the plasma steroid level. During this hour, the calculated half-time of plasma 17-hydroxycorticosteroids was sixty minutes, using the method of least squares to determine the best line for the 3 points.

The response of the plasma 17-hydroxycorticosteroid level to a six-hour intravenous infusion of 25 I.U. of ACTH starting one and a half hours after the end of the previous infusion is shown in Figure 4. These values are indicated by crosses in Figure 5. The rate of increase of the steroid level in Subject D can be seen to lie above the mean values.

Subject E (weight 177 pounds, height 69 inches)

The subject was a 19-year-old, healthy, male volunteer. Day 1 was a control day to observe the normal diurnal variation of the plasma steroid levels (Fig. 6). At 9:00 A.M. on Day 2, a constant infusion of 0.5 I.U. of ACTH per day was started. During this day several specimens were lost, but the maximal and minimal plasma levels observed were about the same as on the control day. On Day 3, the morning and afternoon plasma steroid values showed little change from those of the preceding days. However,

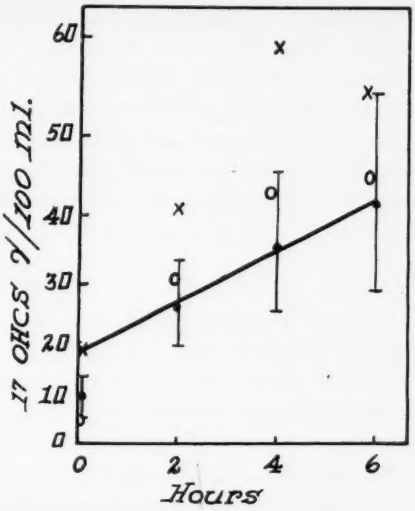

FIG. 5. The mean and standard deviations of the plasma 17-hydroxycorticosteroid levels in 39 normal subjects during infusion of 25 I.U. of ACTH are plotted on an inverse logarithmic scale. Results in *Subject D* are indicated by crosses; those in *Subject E*, by circles.

the level had risen by 8:00 P.M. on the third day, and remained above the control values at 12:00 midnight and 4:00 A.M. It was slightly lower than the control level at 8:00 A.M. on Day 4, when the infusion was discontinued. In another blood sample drawn thirty minutes after ending the infusion there was little change in the concentration of plasma 17-hydroxy-

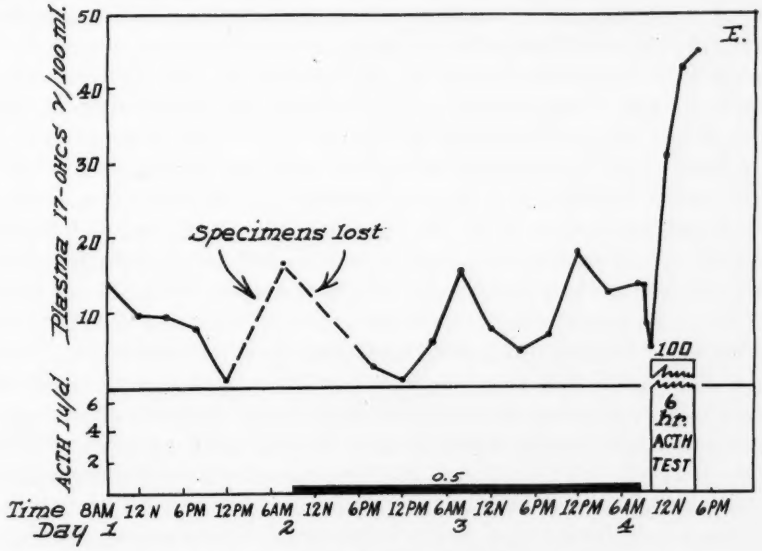

FIG. 6. Study on *Subject E*.

corticosteroids, but there was a rapid fall during the subsequent one-hour period. The half-time of plasma 17-hydroxycorticosteroids during this one-hour period was calculated to be forty-six minutes. One and a half hours after ending the constant infusion, a six-hour intravenous ACTH test was carried out, using 25 I.U. The plasma values are indicated in Figure 5 by circles and can also be seen in Figure 6. The rate of increase of the concentration of plasma steroids in this subject was above the mean (Fig. 5).

DISCUSSION

Several investigators have studied the effects of intravenous administration of small amounts of ACTH on various parameters of adrenocortical function. Bliss, Nelson and Samuels (6), using single intravenous injections of varying amounts of ACTH, found that there was a linear relation between plasma 17-hydroxycorticosteroid concentration two hours after the injection and the log of the dose of ACTH injected. Some of their patients responded to intravenous injection of as small a dose as 0.1 I.U. with a rise in the level of plasma 17-hydroxycorticosteroids. Mandel *et al.* (7) found that clinical remissions in susceptible diseases could be obtained by daily intravenous infusions of 2.5 to 5.0 I.U. of ACTH over 20-hour periods. Cohen and Sulman (8) reported that continuous 24-hour intravenous infusions of ACTH in dosages as small as 1.0 I.U. per day elicited good clinical responses in appropriate cases. DiRaimondo *et al.* (9) concluded from their data that the daily basal production of ACTH by the human pituitary is probably less than 1.0 I.U. a day.

A study of the response of the plasma steroids to small amounts of exogenous ACTH is complicated by the existence of a normal diurnal variation in the plasma concentration of 17-hydroxycorticosteroids. The normal diurnal variation (5) has a pattern similar to that shown on Day 1 in Figure 6. In normal subjects, the average maximum concentration of plasma 17-hydroxycorticosteroids (about 20 γ per 100 ml.) is reached at 6:00 A.M. There is no significant variation of the plasma half-life of 17-hydroxycorticosteroids during the course of the diurnal variation when studied by the infusion of hydrocortisone at different times of the day (10). Therefore, the diurnal variation of 17-hydroxycorticosteroids is due to changes in the rate of production of these steroids and not due to changes in their rates of destruction. Forsham *et al.* (11) reported that the rate of increase of urinary 17-hydroxycorticosteroids was greater after exogenous ACTH given during the day than when the same amount of ACTH was given during the night. We have shown that infusion of 25 I.U. of ACTH intravenously over a six-hour period results in maximal adrenocortical stimulation (1); however, there was a greater increase in plasma 17-hydroxycorti-

costeroid concentration when the infusion was started at 8:00 A.M. than when it was started at 12:00 midnight (10). When subjects were given an infusion of a small amount of ACTH (0.5 I.U.) between 5:00 and 11:00 P.M. and a maximum stimulation test was begun at midnight, there was as great an increase in plasma 17-hydroxycorticosteroid concentration as when the maximally stimulating infusion was started at 8:00 A.M. Under normal conditions, infusions which start at 8:00 A.M. begin just after the adrenal has had a period of endogenous ACTH stimulation of several hours' duration, whereas infusions which start at midnight begin when the adrenal has been relatively inactive for several hours. That is, the diurnal variation in the production of ACTH results not only in a diurnal variation of adrenocortical steroid production, but secondarily in a diurnal variation in the responsiveness of the gland to ACTH.

The half-times of plasma 17-hydroxycorticosteroids determined at the conclusion of the continuous infusions in Subjects D and E (Figs. 4 and 6) were considerably shorter than the half-times observed after large doses (1 mg. per Kg. of body weight) of cortisol (10). However, the values in these 2 subjects were of the magnitude of those determined using tracer amounts of hydrocortisone (12). Since the rates of removal of plasma 17-hydroxycorticosteroids by these subjects were no slower than normal, and since the rise in plasma 17-hydroxycorticosteroid concentration during a six-hour infusion of 25 I.U. of ACTH was greater than the average, the conclusion seems justified that these 2 men responded to the six-hour ACTH infusion with a rate of production of plasma 17-hydroxycorticosteroids above the average. In Subject E, the continuous infusion of ACTH amounted to only 0.5 I.U. per day for a two-day period.

In most of these studies an increasing concentration of plasma 17-hydroxycorticosteroids was found in response to continuous ACTH stimulation, even though the dose of ACTH administered was small by the usual standards and frequently was given in decreasing amounts. These increasing plasma steroid levels presumably were the result of increasing adrenal capacity caused by continuous ACTH stimulation. The effect of a constant infusion of ACTH in causing increased adrenal capacity must be considered when attempting to estimate the rate of secretion of endogenous ACTH. Infusion of ACTH at rates of 2.9, 3.9, 4.8 and 5.8 I.U. per day resulted in elevations of plasma 17-hydroxycorticosteroids within four hours to levels above the physiologic range. The infusions in 3 cases were started between 8:00 and 10:00 A.M., when the subjects' adrenals had just undergone a period of stimulation by endogenous ACTH (10). However, since the period of high endogenous secretion of ACTH is relatively brief, amounting perhaps to four hours, and in every case the subjects promptly attained plasma levels exceeding the maximal normal morning values, it

seems safe to conclude that the normal male can achieve these morning levels by the secretion of ACTH at a rate of less than 4 I.U. per day for a period of four hours (a total of 0.67 I.U.). This rough estimate does not exclude the possibility that the normal adult male may secrete ACTH at a rate of more than 4.0 I.U. per day, transiently, to achieve maximal concentrations of plasma 17-hydroxycorticosteroids in the early morning hours. Also, it is not an estimate of the total amount of ACTH secreted during a day.

In the study on Subject E, maximal and minimal plasma 17-hydroxycorticosteroid levels were not altered by the first day's infusion of 0.5 I.U. of ACTH. This would suggest that this young male subject secreted ACTH at a rate exceeding 0.5 I.U. per day for at least part of the day.

In normal subjects the average plasma 17-hydroxycorticosteroid concentration four hours after beginning a six-hour infusion of 25 I.U. of ACTH is 35 γ per 100 ml. (Fig. 5). In the present study, the 3 normal subjects attained plasma steroid concentrations of 29, 26 and 33 γ per 100 ml. four hours after beginning infusions of ACTH at rates of 4.8, 2.9 and 3.9 I.U. per day, respectively. Since the 3 subjects had plasma steroid levels at the beginning of the infusions which were close to the normal mean, their plasma steroid responses were close to the average maximal response to ACTH. Yet in the subject given an infusion at a rate of 0.5 I.U. of ACTH per day, there was no demonstrable response of the plasma 17-hydroxycorticosteroid level for over twenty-four hours after starting the infusion. There is only a narrow difference in ACTH dosage between no early response of the plasma 17-hydroxycorticosteroids and a nearly maximal response.

The amounts of ACTH given to the subjects in these studies that resulted in marked elevations of the plasma steroid levels are remarkably small, even allowing for increased adrenal capacity due to sustained adrenal stimulation. Elevations of plasma 17-hydroxycorticosteroids above the average maximum levels found during the diurnal cycle were achieved by the infusion of ACTH at rates of 0.8 to 1.4 I.U. per day.

Cessation of the infusion of 0.5 and 1.0 I.U. per day of ACTH was followed in approximately half an hour by a logarithmic fall in the plasma level of 17-hydroxycorticosteroids. Therefore, ACTH in these small doses did not have a prolonged effect on the production of 17-hydroxycorticosteroids outside of causing increased adrenal capacity.

Certain speculations can be made to explain the complex response which we have observed. It may be that there are 2 related effects of ACTH on the adrenal: 1) an immediate increase in hormone production, and 2) a slower action, probably on the production of enzymes involved in the synthesis of 17-hydroxycorticosteroids. The latter effect results in an in-

creased capacity of the gland and, if prolonged, in a measurable hypertrophy. The formation and degradation of intracellular enzymes is a relatively slow process. The delayed effects of ACTH on adrenal capacity fit well with such a concept and contrast with the immediate effect of ACTH on hormone production.

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STUDIES OF ADRENOCORTICAL FUNCTION IN OBESITY

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ABSTRACT

Determinations in 15 normal and 16 obese subjects showed that in the latter the average plasma 17-hydroxycorticosteroid level was below the normal range and the average urinary 17-hydroxycorticosteroid output was higher than normal. The disappearance of injected hydrocortisone sodium succinate was more rapid and the ratio of conjugated to free 17-hydroxycorticosteroids in the plasma was higher in the obese subjects. The increased urinary output of 17-hydroxycorticosteroids, the increased rate of disappearance, and the higher ratio of conjugated to free plasma 17-hydroxycorticosteroid levels may indicate increased production and metabolic removal of adrenal steroids in the obese patient.

INTRODUCTION

IN A previous publication (1) it was suggested that obese people, being under continual mechanical stress, might have increased adrenocortical function. It was shown that results of the intravenous insulin-glucose tolerance test were significantly impaired in nondiabetic obese patients, being comparable to results obtained in patients with proven adrenocortical hyperfunction, or to whom ACTH and cortisone had been administered (Fig. 1).

Newburgh (2) explained the hyperglycemia of obese "diabetics" as a manifestation of obesity, since the hyperglycemia could be controlled by weight reduction. Cohen (3) studied the 17-ketogenic steroid level in obese children before and after weight reduction, and found that it was higher during the obese period than after loss of weight. These authors suggested that overnutrition may produce a functional hyperpituitarism.

As more direct methods of estimating adrenocortical function became available it was felt that accumulated evidence warranted more specific studies along these lines.

MATERIALS AND METHODS

Fifteen normal subjects and 16 obese patients were studied. In 13 (5 normal and 8 obese) only blood and urinary 17-hydroxycorticosteroid determinations were made; in

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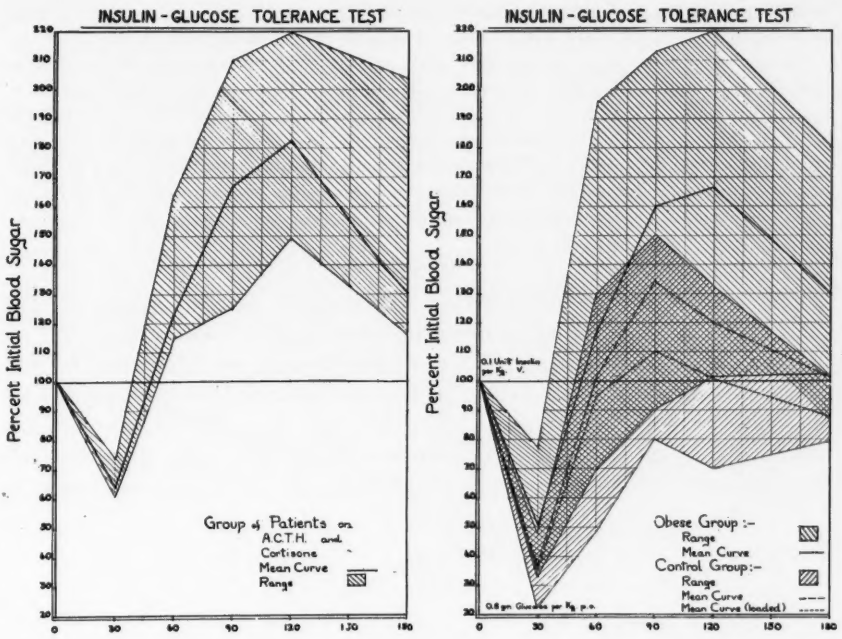

FIG. 1. Insulin-glucose tolerance curves in obese patients and in patients treated with ACTH and cortisone.

18 (10 normal and 8 obese) hydrocortisone disappearance rates were also measured. All of the subjects were ambulatory males, and were selected from the patients and staff of the Queen Mary Veterans Hospital. Two of the obese group were brought into the hospital in order to facilitate the study. No other disease was present and the patients were receiving no medication. A creatinine determination was made on each specimen of urine to check the completeness of the collection. Patients were judged to be overweight when there was a 30 per cent increase according to the ideal weights listed in the Davenport chart.

Plasma 17-hydroxycorticosteroids were determined using the Nelson-Samuels method (4) as described by Harwood and Mason (5); the only modification was that the chloroform extraction of the plasma was performed twice instead of three times. The conjugated corticoids were determined by the method described by Bongiovanni (6).

Urinary corticosteroids were measured by a modification of the method devised by Porter and Silber (7). The urine was incubated at pH5 with β-glucuronidase[1] at 37° C. for twenty-four hours. The mixture was then extracted with chloroform, the extract washed with 1/5 *N* NaOH and water and evaporated to dryness under vacuum. The residue was divided into 2 parts, one serving to develop the color with the phenylhydrazine-sulfuric acid reagent, the other serving as a "urine blank" with sulfuric acid alone. The developed colors were evaluated in a colorimeter with a filter that transmitted light

[1] Ketodase (Warner-Chilcott), 300 Fishman units per ml.

at 415 mμ, reading each sample against its own "urine blank." The amounts of steroid were found from a standard curve obtained with hydrocortisone.

Blood samples were taken at 9.00 A.M., and 24-hour urines were collected from 9.00 A.M. to 9.00 A.M. The plasma clearance of hydrocortisone was studied by the following methods: 100 mg. of hydrocortisone sodium succinate[2] was injected rapidly at 9:00 A.M.; 25 cc. of blood was withdrawn one, two, three and four hours after the injection (with an occasional variation in the time interval). A comparison between the disappearance rate of hydrocortisone sodium succinate and that of the free alcohol of hydrocortisone was made in the same normal subjects. The rates were similar.

RESULTS

The results of the 17-hydroxycorticosteroid determinations in the plasma and urine of normal subjects and obese patients are shown in Figure 2. The normal range of the 9:00 A.M. plasma level in our laboratory is from 6.8 to 15.4 μg. per 100 ml. It can be seen in Figure 2 that the majority of the obese patients had plasma 17-hydroxycorticosteroid levels well below the normal range. Three determinations were in the normal zone; it is felt that some other factor (*e.g.*, thrombophlebitis in 1 patient) was present in

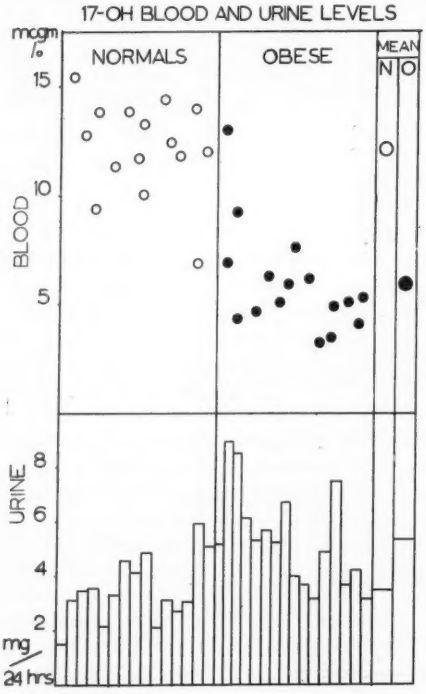

FIG. 2. 17-Hydroxycorticoid levels in plasma and urine of normal subjects *vs.* obese patients.

[2] Supplied as Solu-Cortef through the courtesy of Dr. K. R. Crawley, The Upjohn Company of Canada Limited.

these cases which might explain the atypical results. The mean plasma 17-hydroxycorticosteroid level calculated for the normal persons was 12.14 $\pm$0.60 μg. per 100 ml., whereas that for the obese patients was 5.91 $\pm$0.58 μg. The difference is significant statistically ($P > 0.0001$).

The lower section of Figure 2 shows the 24-hour urinary Porter-Silber 17-hydroxycorticosteroid excretion for the normal controls and the obese patients. The normal range for urinary Porter-Silber 17-hydroxycorticosteroids in our laboratory is 1.6 to 5.9 mg. per twenty-four hours. The mean 24-hour value in the normal persons was 3.49 $\pm$0.40, whereas the mean value in the obese was 5.36 $\pm$0.38 mg. This difference is statistically significant ($P > 0.01$).

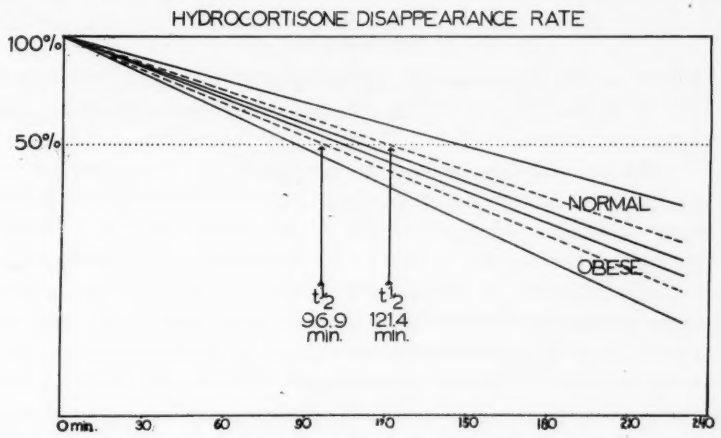

FIG. 3. Half-life of intravenously injected hydrocortisone in normal *vs.* obese subjects.

Hydrocortisone disappearance rate studies were carried out in 18 subjects. The results are shown in Figure 3. The half-life of intravenously injected hydrocortisone (*i.e.*, the time in which the calculated initial 17-hydroxycorticosteroid plasma level declined by 50 per cent) was 121.4 $\pm$2.9 minutes for the normal subjects (from 111 to 148 minutes) and 96.9 $\pm$3.3 minutes for the obese patients (from 87 to 105 minutes). The difference again is statistically significant ($P > 0.001$).

The disappearance rate of hydrocortisone in 2 of the obese patients studied and not included in this series was prolonged. Both had low plasma 17-hydroxycorticosteroid levels and an elevated urinary excretion of Porter-Silber corticoids. These patients are being studied further.

In 4 normal and 5 obese subjects the urinary Porter-Silber 17-hydroxycorticosteroid level was determined at four and twenty-four hours following

intravenous injection of 100 mg. of hydrocortisone. This was done in order to determine whether or not there was any difference in urine level between the 2 groups. No difference was found, and therefore it is assumed that the renal excretion of the steroid was equal in both groups under these circumstances.

In 4 normal and 3 obese subjects, estimation of both free and conjugated plasma 17-hydroxycorticosteroid levels was made. The ratio of free to conjugated in the normal subjects was 1:0.79, whereas in the obese patients it was 1:2.1. Thus the ratio of free to conjugated plasma 17-hydroxycorticosteroid levels was definitely higher in the obese, but the small number of patients and the considerable variation in measurements makes statistical analysis impossible at this time.

DISCUSSION

The observations mentioned in the introduction were interpreted by the investigators as possibly indicating adrenocortical hyperfunction. The low 17-hydroxycorticosteroid plasma levels in the obese (sometimes as low as found in Addison's disease) seemed to contradict this hypothesis. Complementary studies as recorded here were therefore performed. Plasma 17-hydroxycorticosteroid levels alone cannot be relied upon as indicators of adrenocortical function. Effective adrenocortical function is dependent not only on production, but on the length of time the hormones remain in the active form and reach their site of action. A low plasma level together with a low urinary level is usually taken as evidence of decreased steroid production by the adrenal cortex. In the presence of a continuous high urinary steroid output and a low plasma level, some explanation other than a decreased production of steroids must be sought to explain the abnormally low plasma levels. A low plasma level of any substance could be due to:

1) decreased entry (production);
2) greater dilution by increased volume of body fluids or tissues;
3) increased removal by
   a) increased metabolic conversion to a substance not detectable by the test used,
   b) absorption by some body component,
   c) excretion.

A decreased production of 17-hydroxycorticosteroid is very unlikely if not impossible in view of the continuously high urinary steroid output. These findings are compatible with an over-production by the adrenal cortices and a distribution into a larger volume in obese patients than in normal subjects during the diurnal cycle.

Increased metabolic removal must be considered. Results of the studies

on the disappearance from the plasma of exogenous hydrocortisone sodium succinate indicate that the plasma levels decline faster in the obese than in the normal subject. As recorded here, the more rapid drop in the plasma concentration of 17-hydroxycorticosteroids in obese patients could be due to dilution in a greater volume, or absorption by fat depots. However, the increased rate of disappearance remained the same within the whole four-hour period of observation after injection of hydrocortisone, yielding a straight line on semi-logarithmic paper. This is usually interpreted as indicating a first-order metabolic process (8). Considering such an interpretation, it would be rather unlikely that an additional mechanism existed for the removal of hydrocortisone in the obese. On the other hand, if the rate of dilution and increased absorption remains constant during the period of observation, the straightness of the line would not disprove such a mechanism.

Increased excretion in the urine might be due to increased glomerular filtration, diminished tubular reabsorption, or tubular excretion. There is no indication that in obese patients the glomerular filtration rate or tubular excretion are greater than normal; but if such were true, the maintenance of greater excretion of a substance over a period of time must be compensated for by increased availability of the substance; otherwise the body would soon be depleted of it.

The most plausible explanation for the low plasma level, the fast disappearance and the increased urinary excretion would be that the metabolic disposal of the hydrocortisone is more rapid in the obese.

In view of the foregoing factors, it is felt that the increased urinary output of 17-hydroxycorticosteroids with increased rate of disappearance, even in the presence of low plasma 17-hydroxycorticosteroid levels, may indicate increased turnover involving increased production and metabolic removal of adrenal corticoids in the obese patient. It is not felt that this metabolic derangement is an etiologic factor, but it could play a role in the maintenance of obesity or it could simply represent a phenomenon accompanying obesity and maintained by the continuous stress to which obese patients are subject.

Acknowledgment

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PHEOCHROMOCYTOMA IN FATHER AND SON: REPORT OF THE EIGHTH KNOWN AFFECTED KINDRED*

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ABSTRACT

The occurrence of pheochromocytoma of the left adrenal gland in father and son is described, the eighth kindred with familial pheochromocytoma to be reported. A presentation of the family background and distribution of blood groups within the family is included, with the hope that similar studies on other kindred may make linkage calculations and clarification of the mode of inheritance possible. Further genetic implications of chromaffin-cell tumors are noted, as suggested by the frequent occurrence of pheochyromocytoma with neurofibromatosis, the predominance of norepinephrine in chromaffin-cell tumors and the recent findings indicating separate cell origins of norepinephrine and epinephrine.

CURRENT clinical observations on disturbances of the adrenal medulla have been almost completely confined to tumors (pheochromocytomas) producing excessive secretion of catecholamines. The advent of methodologic advances has enabled measurements to be made of catecholamines in body fluids and tissues. These techniques have enlarged understanding of the physiologic role of norepinephrine and epinephrine, and give promise of relating aberrations of catecholamine metabolism and production to human disease states.

Additional evidence of genetic mechanisms involved in the occurrence of pheochromocytoma has been presented by Cone *et al.* (1, 2). These authors recently reported 3 familial cases of chromaffin-cell tumors in 2 unrelated families and reviewed 5 previously recorded instances in which a pheochromocytoma had occurred in more than one member of a given family. We are here describing an instance of pheochromocytoma in father and son. This is the eighth kindred with familial pheochromocytoma to be reported. The genetic pattern is discussed, together with some implications

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of recent findings dealing with adrenomedullary physiology as applied to human genetics.

CASE DESCRIPTIONS

Case 1—L. L., Jr. (Birthdate, June 29, 1954.)

History. This $3\frac{3}{4}$-year-old white boy was admitted to Syracuse Memorial Hospital on May 19, 1958 because of excessive perspiration and elevated blood pressure of four months' duration. He had been in normal good health until four months prior to admission, when excessive perspiration at night was noted, followed by excessive perspiration with minimal exertion. At the same time, an intermittent, slightly raised rash developed over his nose, lasting four to five days at a time. Three and a quarter months before admission, a left exotropia was noted, unaccompanied by visual symptoms. At this time, the patient became easily fatigued, was relatively inactive but still hyperhidrotic even without exercise. Two months before admission, elevated blood pressure was initially recorded. A marked increase in water intake without a corresponding increase in urine volume, and complaints of periodic vague abdominal pain were also noted. The decrease in skin temperature over the fingers and toes became pronounced. No change in behavior or personality was apparent.

The past history was noncontributory. The boy's father had a pheochromocytoma of the left adrenal which was removed at the age of 26 years (see Case 2).

Physical examination. On admission, the patient was a calm, well developed, well nourished, markedly perspiring boy, in no acute distress. He weighed $40\frac{1}{8}$ pounds and was $39\frac{1}{2}$ inches tall. Blood pressures were recorded as follows: right arm supine, 130/88; left arm supine, 130/90; right leg prone, 154/100; left leg prone, 150/94 mm. Hg. Left exotropia was present, without evidence of abnormal innervation. Examination of the ocular fundi revealed generally reduced arteriolar size with a slightly more prominent light reflex than normal. There were no hemorrhages, exudates or arterio-venous nicking. The thyroid could not be palpated. Heart and lungs were normal except for sinus tachycardia and a prominent, forceful apical beat. No masses or organs were visible or palpable in the abdomen; on one occasion, palpation along the mid-line in the right upper abdomen produced a rise in blood pressure to 160/100. The pulse in the femoral vessels was bounding in quality. The dorsalis pedis vessels were just palpable. The fingers and toes were bluish and markedly cold to touch. There was slight puffiness of the dorsum of the hands and feet. An erythematous, slightly raised rash was present over the nose. The testes were descended bilaterally. Neurologic examination yielded normal findings. There was generalized muscular weakness.

Laboratory findings.

1. *General.* The hemogram was normal. Hemoglobin concentration was 13.2 Gm. per 100 ml. The hematocrit reading was 46 per cent. Repeated urinalysis findings were normal. Serum electrolyte levels were as follows: chloride 108 mEq./L, potassium 4.9 mEq./L, sodium 149 mEq./L, and carbon dioxide 23 mEq./L. The blood urea nitrogen level was 19 mg. per 100 ml., and fasting blood sugar concentrations on two consecutive days were 100 mg. amd 85 mg. per 100 ml. The serum protein-bound iodine level was 4.7 μg. per 100 ml. The electrocardiographic pattern was within normal limits for the patient's age. X-ray films of the wrists revealed a bone age of 2 years 8 months compared to a chronologic age of 3 years 9 months. A chest film was normal. A flat plate of the abdomen did not show any calcifications or soft-tissue masses.

2. *Determination of catecholamine levels in blood and urine.* As shown in Table 1, preoperative levels of norepinephrine in both urine and plasma were markedly elevated.

TABLE 1. PREOPERATIVE AND POSTOPERATIVE CATECHOLAMINE LEVELS IN URINE AND PLASMA, CASE 1*

	Plasma		Urine	
	Epinephrine	Norepinephrine	Epinephrine	Norepinephrine
Case 1, preoperative	0 μg./L	53 μg./L	32 μg./day	930 μg./day
Case 1, postoperative (5 days)	2.9 μg./L (total catecholamines)		19 μg./day (free catecholamine, expressed as norepinephrine equiv.)	
Normal adults	epinephrine: 1 μg./L norepinephrine: 3–6 μg./L		norepineph.+epineph., 10–75 μg./day in a ratio of approx. 3:1	

* These determinations were performed by Dr. A. Sjoerdsma, Section on Experimental Therapeutics, National Heart Institute, National Institutes of Health.

No epinephrine was found in the plasma, and the urinary level was only slightly increased.[1]

3. *Pharmacologic data* (*Fig. 1*). After 2 cold pressor tests, which showed elevations of systolic blood pressure of 35 mm. and 20 mm. within two minutes, a somewhat irregular baseline was established over a ten-minute period. Following this, an intravenous dose of 2 mg. of phentolamine hydrochloride (Regitine®) was given rapidly. A

[1] Determination of plasma and urinary 17-hydroxycorticoids and urinary 17-ketosteroids before and after surgery showed no abnormalities, and no significant difference between preoperative and postoperative levels.

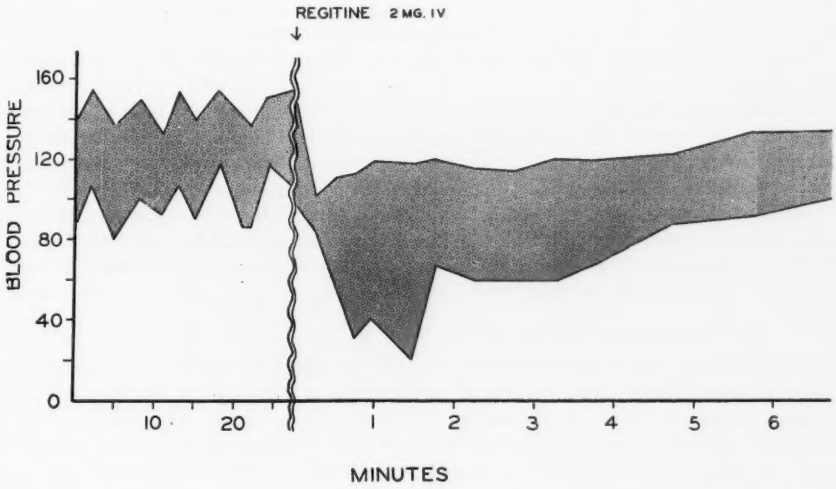

FIG. 1. Response to intravenous phentolamine (Regitine®), *Case 1*.

marked fall in systolic and especially in diastolic pressure ensued, reaching a maximum two minutes after injection, and slowly returning to normal over an eight-minute period. In view of this fairly clear-cut response, no additional tests were performed.

4. *Radiologic attempts at localization of the tumor.* An intravenous pyelogram demonstrated normal-appearing renal calyces, pelves and ureters bilaterally. The axis of the left kidney was more perpendicular than normal, suggesting a mass medial to the left kidney. Subsequently, presacral injections of carbon dioxide were made on consecutive days—initially without, and then with simultaneous opacification of the collecting systems with an intravenous pyelogram. The final examination showed good delineation of the left kidney and partial delineation of the right. A dense soft-tissue area at the apex of the left kidney was visualized, but could not be definitely identified as a specific tumor (Dr. A. S. Berne).

Preoperative course. The blood pressure ranged between 130/90 and 180/110 mm. Hg during the first three days following admission. When the diagnosis became established, phentolamine (Regitine®) was started in a dosage of 12.5 mg. orally every four hours, beginning five days before surgery. This was increased to 12.5 mg. every two hours three days before surgery. Following the increased dosage the blood pressure did not fall to normal but remained between 110/65 and 150/110. The fingers and toes became warmer and perspiration decreased markedly following institution of oral therapy.

Operative course. Seven days following admission, a laparotomy was performed by Dr. Lawrence K. Pickett through a transverse transabdominal incision. Seconal (60 mg.), scopolamine (0.2 mg.) and phentolamine hydrochloride (2 mg.) were given intramuscularly one hour prior to surgery. Anesthesia was maintained with ether following induction with nitrous oxide. An encapsulated mass measuring 3×3×4 cm. was found lying medial to the left adrenal. Thorough exploration of the opposite adrenal and the para-aortic region did not reveal any additional masses. Following ligation of the vein leading from the tumor, the blood pressure fell abruptly to zero, but was restored to normal with levarterenol (Levophed®) intravenously. Beginning thirty minutes following removal of the tumor, the patient's blood pressure was maintained at normal levels or above without levarterenol. For the first three days following surgery, the blood pressure remained slightly elevated at approximately 125/90 mm. Hg, subsequently falling to values between 110/70 and 120/80. The postoperative period was completely uneventful.

Histologic findings. The tumor was an encapsulated round mass weighing 7.65 Gm. (Fig. 2). Histologically, its characteristics were diagnostic of a benign pheochromocytoma. The tumor contained 4.4 mg. of norepinephrine per gram of tissue. There was no detectable epinephrine (less than 10 per cent of the norepinephrine value).

Subsequent course. Plasma and urinary catecholamine levels were within normal limits five days postoperatively. The patient was discharged from the hospital on April 3, 1958, sixteen days after admission. Follow-up monthly visits for four months have revealed a healthy, normotensive boy.

Case 2—L. L. (29 year-old father of L. L., Jr., Case 1).

History. This white man was first admitted to this Medical Center on September 10, 1955 at the age of 26 years. He entered the hospital with the chief complaint of left-sided weakness of a half day's duration. On the night preceding admission, after retiring, he noted numbness of the left hand and arm. Awakening at 3 A.M. to void, he found that his left arm and leg were weak and numb; he walked with a limp and was barely able to move his left arm. Elevated blood pressure was first recorded in this

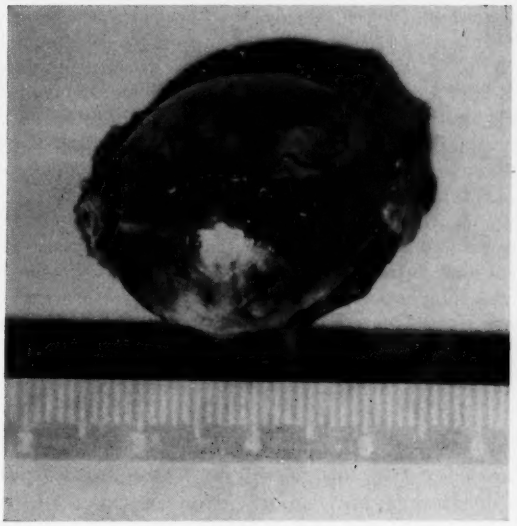

FIG. 2. Pheochromocytoma of left adrenal photographed immediately following operative removal, *Case 1*.

patient when he was in service in Korea in 1952. Because of this he was returned to the United States and hospitalized, but no diagnosis was established. He was subsequently given a medical discharge. Medical follow-up was sporadic from that time until his 1955 admission. He had occasional occipital headaches and several "black-out" spells, without complete loss of consciousness. Excessive perspiration and easy fatigability had been present for the preceding several years.

Physical examination. On admission, he presented as a well developed, well nourished, lethargic man in no acute distress. Blood pressure was 150/130 mm. Hg, supine. Funduscopic examination revealed narrowed arterioles and increased tortuosity of the veins. The thyroid gland was not palpable. The heart was not enlarged; a grade III blowing systolic murmur was present at the apex, transmitted into the axilla. No organs or masses could be palpated in the abdomen. Peripheral pulses were present and equal. Neurologic examination revealed left facial weakness with ptosis of the left upper lid, and slight weakness of the left hand and arm. No abnormal reflexes were present.

Laboratory findings. The hemogram was normal. Results of urinalyses were negative. Serum chemical determinations revealed the following levels: nonprotein nitrogen, 45 mg. per 100 ml., fasting (blood) sugar 113 mg. per 100 ml., sodium 137 mEq./L, potassium 3.7 mEq./L, chloride 92 mEq./L and carbon dioxide 32 mEq./L. During a Thorn (ACTH) test the eosinophil count dropped more than 50 per cent.

The cerebrospinal fluid was normal. Urinary catecholamines were determined in the laboratory of Dr. M. Goldenberg of Columbia University and were reported as "significantly elevated, diagnostic of pheochromocytoma." An intravenous pyelogram showed normally functioning kidneys. Retroperitoneal injection of oxygen delineated a sharply circumscribed mass measuring 5 cm.×5 cm. in the region of the upper pole of the left kidney.

Preoperative course. Following admission, the blood pressure varied from admission levels to 240/150. Intravenous injection of 5 mg. of phentolamine (Regitine®) four times produced significant falls in the blood pressure within one minute; similarly the intravenous injection of 18 mg. of benzodioxane intravenously produced a significant drop, starting thirty seconds after beginning the injection and reaching a maximum at two and a half minutes. Because of intermittent dangerously high blood pressure levels, attempts were made to prevent recurrent cerebrovascular accidents, using intramuscular and intravenous phentolamine.

Operative course. On October 20, 1955, through a thoraco-abdominal incision, a lemon-sized tumor was found in the left adrenal medulla. It was necessary to administer levarterenol by constant intravenous drip for two days postoperatively. The postoperative course was complicated by development of atelectasis and left hemothorax. The ultimate course was satisfactory and the patient remains asymptomatic two and three-quarter years after removal of the tumor.

DISCUSSION

The concepts elaborated by Cannon (3) with respect to the role of the adrenal medulla in the response to a wide variety of stimuli have undergone considerable change and modification in the past ten years. Von Euler and Hamberg (4) demonstrated that the medulla elaborated norepinephrine in addition to epinephrine, and that norepinephrine was the principal sympathomimetic substance present in adrenergic nerves (5). As a result of these discoveries and the refinement of knowledge regarding the physiologic effects of norepinephrine and epinephrine, it has become apparent that the function of the adrenal medulla cannot be defined in terms of accentuation of its sympathetic innervation, for in some respects the two are antagonistic (6).

The clinical recognition of tumors secreting excessive amounts of catecholamines is being accomplished with greater frequency and preciseness (7–9). Refinements of pharmacologic methods for either blocking or stimulating adrenomedullary secretions (10–12) and radiologic methods for localization of suspected pheochromocytomas (7, 13) have proved of considerable aid. With the demonstration of strikingly dissimilar as well as similar physiologic effects of epinephrine as opposed to norepinephrine (6, 13, 14), it might be supposed that the type of hormone being elaborated by a hyperfunctioning tumor could be recognized clinically. Such a clinical differentiation, however, may be misleading because of the occurrence of both hormones in many tumors, as well as the paradoxical physiologic effects of large doses of either hormone (15).

GENETIC IMPLICATIONS

1. *Familial cases.* In the reports by Cone *et al.* and others (1, 2, 16–20), varying patterns of occurrence combined with relatively limited investigations of the family backgrounds make it difficult to draw conclusions as to

the possible existence of a common mode of inheritance. In our case, except for the father, no additional cases of pheochromocytoma were uncovered in the family tree (Fig. 3). A paternal aunt (#3 in Fig. 3) died at the age of 48 from a cerebrovascular accident after having severe hypertension and recurrent "nervous breakdowns" for many years; no autopsy was performed. Two other persons had hypertension, not confirmed, but had no other symptoms suggestive of pheochromocytoma.

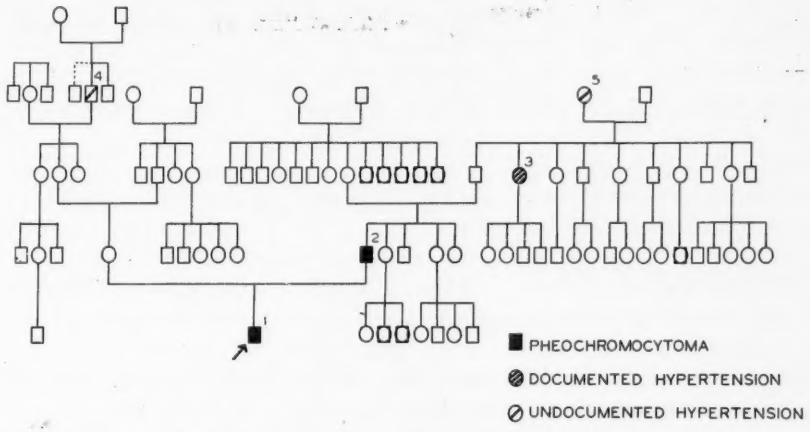

FIG. 3. Pedigree of family discussed in this report (see text)

The distribution of blood groups within the family that we are reporting is shown in Table 2. Although no conclusions can be drawn at present from these data, it is hoped that similar studies on other kindred with this tumor may make linkage calculations possible.

2. *Relationship to neurofibromatosis.* Numerous reports have implicated the rather striking incidence of neurofibromatosis in association with tumors of chromaffin tissue (21–28). The common embryologic origin of chromaffin tissue and tissue of neural origin has been noted by many workers (29–31). Cells destined to become chromaffin tissue cannot be differentiated from other cells of neural origin in the sympathetic primordia in early embryos. Displacement of cells from adjacent sympathetic primordia into the adrenal capsules occurs in human embryos at the 15-mm. stage, and well organized chromaffin bodies are not found until the 30-mm. stage. Neurofibromatosis, although usually involving peripheral nerves, may occur within the autonomic nervous system (29). A dominant mode of inheritance of neurofibromatosis has been clearly described in the studies of Crowe *et al.* (32) and Borberg (33). A lack of heterogeneity in the clinical material of

TABLE 2. BLOOD GROUPS OF FAMILY DISCUSSED IN THIS REPORT
(Note: Antiserum for d not available)

Patient (Case 1)	A_1	MNss	$\frac{DCe}{dce}$ or $\frac{DCe}{Dce}$	Duffy pos.	Kell neg.
Father (Case 2)	A_2	MNss	$\frac{DCe}{dce}$ or $\frac{DCe}{Dce}$	Duffy pos.	Kell neg.
Mother	A_1	MNss	$\frac{DCe}{DCe}$ or $\frac{DCe}{dCe}$	Duffy pos.	Kell neg.
Paternal grandmother	A_1	MNss	$\frac{DCe}{dce}$ or $\frac{DCe}{Dce}$	Duffy neg.	Kell neg.
Maternal grandmother	A_1	MNss	$\frac{DCe}{DCe}$ or $\frac{DCe}{dCe}$	Duffy pos.	Kell neg.
Maternal grandfather	A_1	MNss	$\frac{DCe}{dce}$ or $\frac{DCe}{Dce}$	Duffy pos.	Kell neg.

Crowe *et al.* led them to the conclusion that probably only one locus was involved in the genetic transmission.

A correlation between the sporadic cases of neurofibromatosis and our case is of interest. Crowe *et al.* noted that when dealing with a dominantly inherited disease occasioned by a gene with a high level of penetrance, it is customary to attribute the occurrence of sporadic cases of the disease to mutation. The assumption of mutation can be made, according to these authors, when the following are present: 1) the offspring of sporadic cases are affected and in a ratio compatible with dominant heredity; 2) there exists no evidence of an effect of maternal age and/or parity on the distribution of sporadic cases; and 3) the sporadic case is clinically indistinguishable from the familial. Although the cases in the present study may superficially fill these requirements, the elucidation of the genetic pattern of familial pheochromocytoma requires further documentation. (See Addendum, page 359.)

3. *Nature of hormones produced by pheochromocytomas.* Since norepinephrine is the predominant sympathomimetic substance found in adrenergic nerves, a possible relationship between tumors of chromaffin cells and tumors involving tissue of neural origin may be deduced by the predominance of norepinephrine in almost all pheochromocytomas that have been differentially analyzed for both amines (34–36), as exemplified by Case 1. Tumors of extra-adrenal chromaffin tissue produce norepinephrine exclusively,

whereas those of the adrenal medulla often elaborate both amines, with levels of norepinephrine usually far exceeding those of epinephrine.

There is a stage (until the end of the first year of life) in the development of the human being as well as of almost all animals studied, when norepinephrine is the predominant hormone secreted by the adrenal medulla (37–40). Whereas there are significant differences in the relative percentages of both amines in the adrenal medulla among different species, there is a definite similarity of distribution and maturation of both catecholamines within any given species. In addition, West *et al.* (41) have shown that extra-adrenal chromaffin tissue, as exemplified by the organ of Zuckerkandl, develops structurally and functionally to a much greater extent during fetal life than does the adrenal medulla. Thus, during the first year of life of the human infant, the extra-adrenal chromaffin tissues contain greater amounts of catecholamines (again largely norepinephrine) than does the medulla, and appear to be the main source of pressor amines during that period of life. The factors accounting for the dissolution of extra-adrenal chromaffin tissue or for the maturation of epinephrine production by the adrenal medulla remain unclarified.

In relating the maturation process described to the problem of familial pheochromocytoma, it would be important to know if both norepinephrine and epinephrine are produced by similar or by different cells. Bülbring and Burn (42) obtained evidence of the conversion of norepinephrine to epinephrine in perfused canine suprarenals by observing the disappearance of norepinephrine and a corresponding increase in the amount of epinephrine in the perfusate. Similarly, others have confirmed the role of norepinephrine as an intermediary in epinephrine production (43). However, if it be assumed that both hormones are produced by the same cell and that the proportions of the two hormones are dependent on the degree of methylation of norepinephrine, several features are not easily explained. As pointed out by von Euler (44), "such a graded activity of an enzyme system present in all cells, as it were, would require a regulation system of extraordinary accuracy." Indirectly, it has been demonstrated, for example, that insulin provokes epinephrine secretion, whereas norepinephrine secretion is secondary to stimulation of certain hypothalamic areas (45). More directly, Hillarp and Hökfelt (46, 47) demonstrated differential staining of norepinephrine-producing cells, and feel that a specific cell type produces each hormone. It will thus be of importance if a relationship can be established between pheochromocytoma and "specific" norepinephrine-producing cells.

Addendum

A requirement for assuming spontaneous mutation in sporadic cases of a given abnormality need not be the absence of evidence of an effect of maternal age, as previously

suggested under "Genetic implications." As indicated by James V. Neel (*Pediatrics* **19**: Part II, 749–754 (Apr.) 1957), the variety of causes of so-called spontaneous mutation in man should be expected to be cumulative in action, or show an "age effect."

Acknowledgments

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TREATMENT OF A LINGUAL GOITER WITH TRIIODOTHYRONINE

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ABSTRACT

This report concerns a myxedematous patient, aged 55, with severe dysphagia produced by a large vascular tumor at the base of her tongue. Radioiodine was useful in establishing a diagnosis of lingual goiter. However, in dosages which could be tolerated by the patient, it could not be concentrated sufficiently for therapeusis. Since the gland was too vascular and large for easy surgical removal, the patient was given 75 μg. of triiodothyronine per day, both for treatment of the myxedema and for shrinkage of the goiter via suppression of pituitary thyrotropic activity. The goiter shrank remarkably within six weeks and the patient became asymptomatic. Desiccated thyroid was then substituted as maintenance therapy. Such treatment is recommended: 1) as preoperative preparation when surgery is necessary, or 2) as the sole treatment of large lingual goiters.

CONGENITAL anomalies in the location of thyroid tissue are not unusual; part or all of this gland may occur in sites ranging from the base of the tongue to the myocardium. The most common location for aberrant thyroid tissue is in the region of the tongue. A lingual thyroid occurs when the central anlage of the gland fails to migrate from its site of origin as an outcropping of the first pharyngeal pouch and develops on or in the posterior portion of the tongue. Several such cases are reported in the literature each year, and excellent reviews are available (1–9). Less commonly, the thyroid anlage as it migrates down the neck may leave remnants along the way, or it may migrate too far, finally lodging in the thoracic cage. Lingual thyroid tissue often remains completely unnoticed for years, only becoming apparent when a goiter develops.

The diagnosis and treatment of such aberrant tissue is difficult. Deforming surgical procedures have been necessary when the normal anatomic relationships were disturbed by ectopic tissues. The introduction of the radioactive iodine tracer technique has made the diagnosis of these anomalies simpler and safer (10–12). Several cases of lingual thyroid with symp-

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toms of dysphagia, obstruction and hoarseness have been treated with radioiodine (I^{131}) in the past eight years; the radiation necrosis results in scarring and reduction in size (11–13). However, in treating hypothyroid patients who have large aberrant goiters, radioiodine may not be advisable, for the low radioiodine uptake in such tissue may necessitate a larger dose than the patient can safely tolerate.

We have recently seen a patient with a lingual goiter in whom neither a biopsy nor surgical removal was feasible because of the extreme vascularity and size. The diagnosis was established with the aid of radioiodine, but treatment with this material would have required a tremendous dose. Accordingly, she was treated with triiodothyronine in an attempt to reduce the size of the gland.

CASE REPORT

A 55-year-old woman had been a resident of a state institution for the feebleminded in the State of Washington for twenty years. No other difficulty was known until about three months prior to admission, when it was first noted that she had difficulty swallowing solid foods, and that her voice had a nasal sound. These symptoms progressed until finally a large tumor was seen to be occluding the pharynx. For these reasons she was referred to the King County Hospital surgical service.

Physical examination revealed a pale white female of short stature, who spoke with a marked nasal twang. Blood pressure was 140/80, and pulse 80. The positive findings included dry, slightly thickened skin, coarse scalp hair, and only small amounts of axillary hair. The pharynx was almost completely occluded by a symmetrical, soft, pink tumor which rose from the posterior third of the tongue (Figs. 1 and 2). The posterior pharynx and uvula could not be seen by direct visualization. A complete oral examination[1] showed that the tumor extended posteriorly almost as far down as the epiglottis. There was no palpable thyroid in the usual location in the neck. The rest of the findings on physical examination were negative.

With the possibility in mind that this was a cystic tumor, aspiration was attempted with a No. 21 needle. No fluid could be obtained, and there was loss of some blood, with continual oozing for two to three hours. Because of this extreme vascularity and the large size of the goiter, surgical biopsy or removal would have been difficult.

Further studies revealed a serum protein-bound iodine level of 2.2 micrograms (μg.) per 100 ml. (normal, 3.5–8.0 μg.), and a thyroidal radioiodine (I^{131}) uptake of 5 per cent in twenty-four hours. By using a deep lead cone with a field of only 2–3 sq. cm., it was possible to demonstrate that there was no iodine uptake in the normal thyroid area, and that the only increased uptake was in the area of the tumor mass. It was concluded that this was a lingual thyroid gland, and that it was the only functioning thyroid tissue in the body.

The patient was given 75 μg. of triiodothyronine daily, a dosage which made her mildly thyrotoxic. This was continued for six weeks, when re-evaluation was made. The gland had shrunk remarkably and was no longer immediately visible on examination of the oral cavity. The posterior pharynx and uvula were easily seen. The small residual mass of tissue, which could be elevated into view by stimulation of the gag reflex, is

[1] Kindly performed by Dr. John Gehrig, Executive Officer of the Oral Surgery Department, University of Washington School of Dentistry.

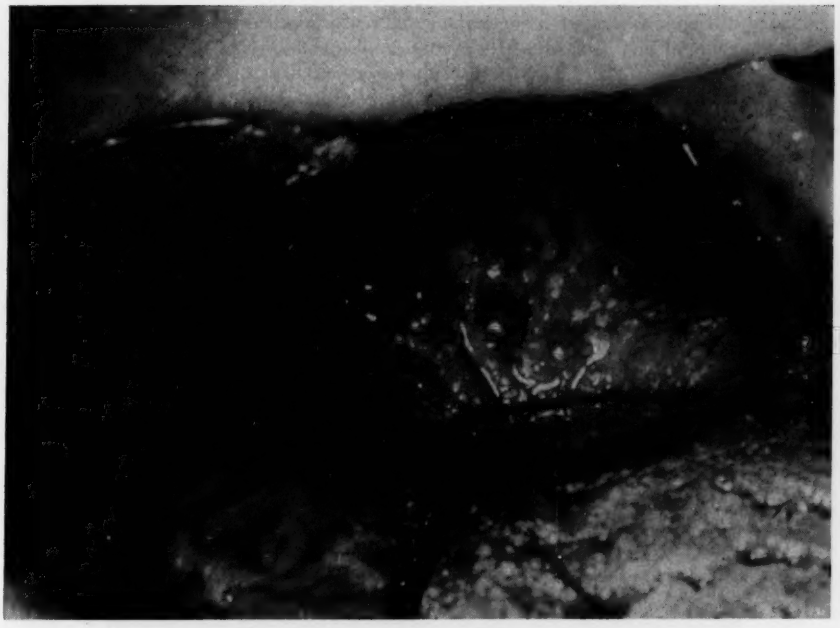

FIG. 1. Photograph taken before treatment, showing the pharynx almost entirely occluded by a large, vascular, lingual thyroid. The posterior pharynx cannot be seen.

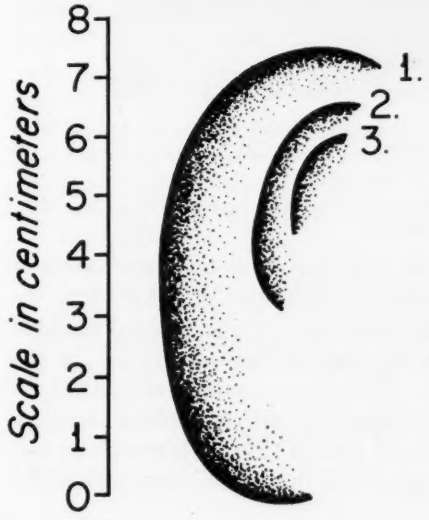

FIG. 2. Reduction in size of lingual thyroid during treatment with triiodothyronine and desiccated thyroid. Size estimated from x-ray examination and direct visualization. The shaded areas represent the posterior border as seen from a lateral view: 1) before treatment, 2) after six weeks of treatment, and 3) after four months of treatment.

illustrated in Figure 3. At two months the gland had atrophied further, and medication was changed to 90 mg. of desiccated thyroid daily for maintenance. No other type of treatment seemed necessary, and the patient was released to the state institution.

At the time of follow-up examination four months later the gland had shrunk further (Fig. 2). A radioiodine uptake test showed no retention in the head and neck area, and there were no symptoms referable to the lingual thyroid. The patient was still mildly thyrotoxic and therefore the dosage of desiccated thyroid was reduced to 60 mg. per day.

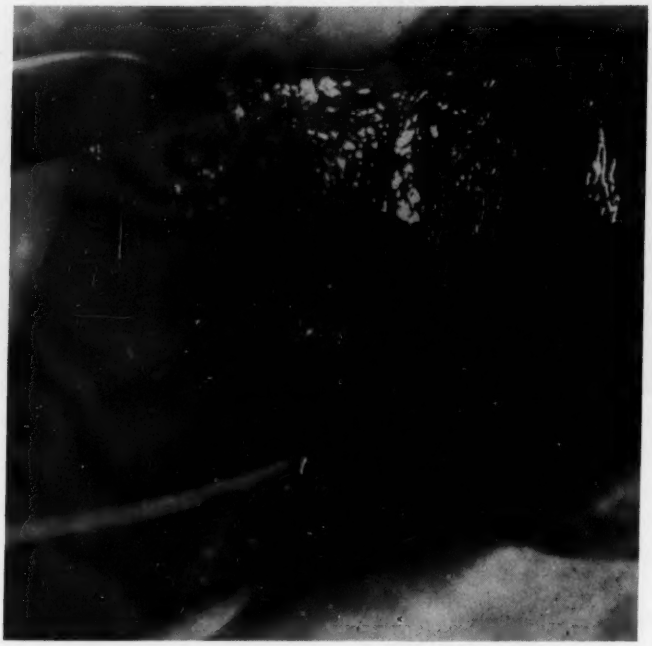

FIG. 3. After six weeks of treatment with triiodothyronine. Unless the tongue were depressed as shown, direct visual examination of the throat would not reveal the remnant of a lingual thyroid.

DISCUSSION

Because of the low iodine uptake of this lingual goiter, large doses of radioiodine would have been necessary for treatment. Furthermore, the large size and vascularity of this gland would have made surgical removal difficult and dangerous. In most reported cases of lingual thyroid, the patients had no other thyroid tissue (1–9). Thus, they would have had to take thyroid hormone for the rest of their lives if the aberrant gland were destroyed. Our patient was hypothyroid and needed thyroid hormone to correct this abnormality. Moreover, we suspected that a sufficient dose might

suppress completely the release of thyrotropic hormone from the pituitary (14), and might reduce the size of the lingual goiter. Accordingly, she was treated with triiodothyronine, which markedly inhibits release of thyrotropic hormone (15). This resulted in pronounced atrophy of the lingual gland, with complete relief of symptoms. Medication was then changed to desiccated thyroid, the dose being regulated to maintain the patient in a euthyroid state.

To our knowledge this is the first case of a lingual thyroid goiter with symptoms of obstruction that has been intentionally treated in order to suppress production of thyrotropin. In 1935 Montgomery (7) treated a lingual goiter with a saturated solution of iodine (Lugol's), and over a 73-day period noted remarkable shrinkage of the gland. Unfortunately, the patient was lost to follow-up. In 1950, Crispell and Parson (12) reported a case of lingual goiter in a patient thought to be mildly hypothyroid, whom they treated with 6.8 millicuries of I^{131}. The patient was given desiccated thyroid in a dosage of 1 grain daily, to correct the mild hypothyroid state. In a three-month follow-up there was no objective evidence of thyroid atrophy. Six months later the gland was smaller. Radioiodine usually results in a maximum reduction in gland size in about six weeks, and the prolonged time lag in this case suggests that the atrophy may have been at least partially attributable to treatment with thyroid hormone rather than to radioiodine.

The advantages of suppression of thyrotropin as a means of treatment in these patients, who usually must take thyroid anyway, seem obvious. Both the operative danger itself and the possibility of scarring of the tongue by surgery or radioiodine are eliminated. If surgery becomes necessary, preoperative treatment with triiodothyronine will greatly reduce the size and vascularity of the gland, making either a surgical biopsy or removal simpler and safer.

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INACTIVATION OF THE ANTIDIURETIC ACTIVITY OF VASOPRESSIN DURING PREGNANCY: A REPORT OF A CASE*

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ABSTRACT

A case is reported in which severe diabetes insipidus occurred following hypophysectomy performed for carcinoma of the breast in a pregnant patient. During pregnancy, the diabetes insipidus could not be controlled by relatively large doses of vasopressin. Following parturition, the necessity for administration of vasopressin (Pitressin) ceased by the ninth day.

A study is described in which a physiologic dose of vasopressin was administered intravenously under standard conditions of water diuresis in the pregnant and in the postpartum periods. The shorter duration of negative free water clearance that was noted during pregnancy is attributed to the destruction of vasopressin at an increased rate. Presumably, endogenous antidiuretic hormone was being similarly destroyed. It is suggested that this is due to a circulating enzyme elaborated by the placenta. Recurrence of the polyuria was noted in the preterminal period. This could readily be controlled by the administration of vasopressin.

INTRODUCTION

A DEFICIENT production of antidiuretic hormone by the hypothalamico-hypophysial system results in polyuria, polydipsia and the secretion of urine of low osmolar concentration. This deficiency may follow damage to the centers of antidiuretic hormone formation from trauma, neoplasm, sepsis or some vascular abnormality. Furthermore, it is conceivable that increased peripheral inactivation may produce a relative lack of this principle.

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In diabetes insipidus due to deficient antidiuretic hormone, the sensitivity of the renal tubules to this substance can be demonstrated by means of the intravenous injection of vasopressin during water diuresis. This is followed by a decrease in the rate of urinary flow, and a concomitant increase in urinary osmolarity.

In the case to be described, large amounts of exogenous vasopressin (Pitressin R; Parke Davis & Co.) failed to control the symptoms of diabetes insipidus during pregnancy, despite sensitivity of the renal tubules to this material.

CASE REPORT

D. de H. (PBBH #3K964), a 37-year-old gravida 4 para 3 white female, was first admitted to the Peter Bent Brigham Hospital on December 26, 1956. At the time of admission, the patient was twenty-three weeks pregnant. One month prior to admission, biopsy had revealed carcinoma in the left breast and the left axillary lymph nodes. During previous pregnancies, there had never been any toxemia, polyuria or polydipsia.

In the preoperative period, with limited fluid intake, urine volumes averaged about 800 ml. per day. Hypophysectomy was performed on January 7, 1957, with minimal trauma to the hypophysial stalk. Diabetes insipidus developed in the immediate postoperative period, with urine volumes reaching 13,000 ml. on the first postoperative day. Cortisone therapy, begun on the day prior to operation, was gradually reduced to a maintenance level of 75 mg. per day. Thyroid substitution was not started for one month, after which a maintenance dose of 90 mg. per day was administered.

Vasopressin snuff was begun on the fifth day after operation, with little effect. After two weeks of administration, allergy to the snuff developed; this was manifested by asthmatic attacks which stopped with cessation of snuff therapy. One unit of vasopressin tannate in oil was started on the eighteenth postoperative day and given every second day. This did not control the polyuria or the polydipsia. Progressively larger doses of vasopressin tannate in oil were given, but even a dosage of 3 units every second day, supplemented by aqueous vasopressin, failed to reduce the daily urinary volumes or the thirst of the patient. Alteration of the suspending vehicle from peanut oil to sesame oil did not improve the efficacy of the medication.

Labor was induced by membrane rupture on March 14, 1957, and completed on the following day by the spontaneous delivery of an infant weighing 5 pounds 1 ounce. The infant did not exhibit signs of diabetes insipidus.

Gradually decreasing doses of vasopressin were administered for nine days following delivery. The requirement for this therapy then disappeared. Cortisone dosage, elevated during labor and on the first postpartum day, was rapidly returned to the maintenance level of 75 mg. per day.

On the eleventh postpartum day, there developed the first signs of the respiratory disease which was to prove fatal four weeks later. These signs were accompanied by sporadic temperature elevations. Three days later, cortisone therapy was increased to 150 mg. per day. Daily urine volumes subsequently rose to levels of 5,000 ml. At this time, administration of aqueous vasopressin satisfactorily controlled the nocturnal polyuria and polydipsia. The patient expired on April 12, 1957.

Microscopic examination of the placenta failed to reveal any significant abnormality. At autopsy, no pituitary tissue was found in the sella turcica. Renal morphology was normal.

DISCUSSION

The coexistence of diabetes inspidus and pregnancy is an infrequent occurrence, but has been described (1–3). Two of the patterns of this disease during pregnancy are the temporary exacerbation of pre-existing diabetes insipidus or the appearance of a transient polyuria and polydipsia with disappearance following parturition. These patterns have a common feature, namely, the polyuria and polydipsia are worse during pregnancy and improve after parturition.

The case which has been described, in which a severe iatrogenic diabetes insipidus commencing in pregnancy improved after delivery, also had this characteristic. During pregnancy, relatively large doses of vasopressin failed to control the symptoms. After delivery, the need for vasopressin diminished, and by the ninth postpartum day had disappeared.

These observations may be explained in the following way. Prior to hypophysectomy, the secretion of antidiuretic hormone and the response of the kidneys to it were normal. This was shown by the patient's ability to limit urinary excretion to small volumes when fluid intake was restricted.

Damage done to the cells of the hypothalamic area during hypophysectomy led to decreased production of antidiuretic hormone. Diabetes insipidus ensued. Random determinations of urine osmolarity and creatinine excretion before parturition showed that this polyuria was not an obligatory osmotic diuresis in the presence of maximal antidiuretic hormone activity.

In the period between hypophysectomy and delivery, when the diabetes insipidus was most severe, it may be assumed that some endogenous antidiuretic hormone production was present. Evidence for this is indirect, but is seen in the ability of the patient in the postpartum period to control water excretion without needing added vasopressin.

However, despite this endogenous antidiuretic hormone and the added vasopressin, severe diabetes insipidus persisted, abating only following delivery. The daily urine volumes and vasopressin therapy are shown in Figure 1.

In an attempt to clarify the picture of the diabetes insipidus and of the vasopressin requirements in this patient, the effect of intravenous Pitressin was studied in the thirty-fifth week of pregnancy and on the thirteenth postpartum day. Similar conditions of water loading, food intake, cortisone and thyroid therapy were observed on each occasion. The results of these tests are shown in Figure 2.

In the test performed during pregnancy, intravenous injection of 20 milliunits of vasopressin after initiation of water diuresis produced hypertonicity of the urine and negative free water clearance for a short period.

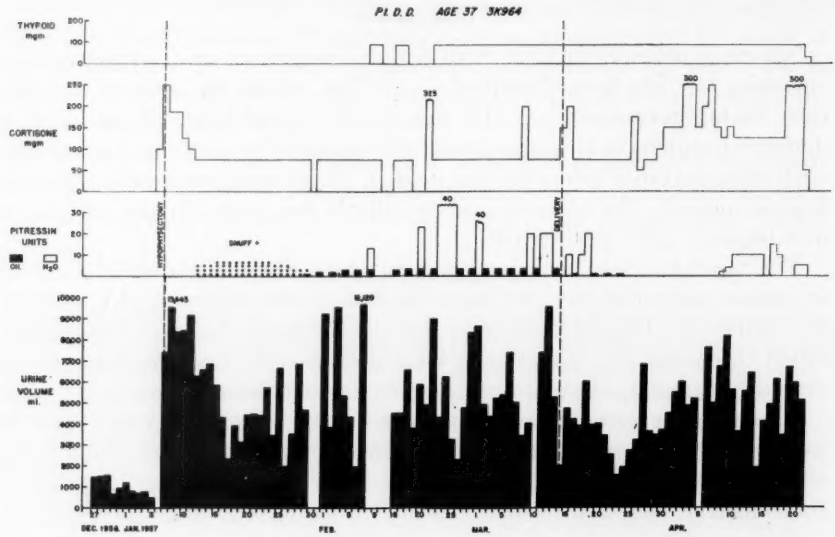

FIG. 1. Urine volumes, and dosages of vasopressin, cortisone and thyroid during pregnancy before hypophysectomy, and also following hypophysectomy before and after delivery.

In the postpartum state, under similar conditions, a prolonged period of antidiuresis and greater negative free water clearance with increased urinary osmolarity were seen following intravenous injection of the same amount of vasopressin.

The duration of antidiuretic effect following the intravenous injection of a single dose of vasopressin into a subject with established water diuresis can be related (4) to the administered dose. Presumably, repeated injection of the same dose should produce similar periods of antidiuresis.

The prolonged antidiuresis following intravenous injection of vasopressin during the postpartum period as compared with that during pregnancy suggests that the amount of antidiuretic material reaching the renal tubules was greater in the former than in the latter period. Since the same dosage of vasopressin was administered on each occasion, inactivation of both exogenous and endogenous vasopressin was probably occurring during pregnancy.

The purpose of this paper is to present these observed facts. There are several possible mechanisms which may have accounted for the amelioration of the diabetes insipidus in the postpartum period and the alteration in response to a standard dose of vasopressin before and after delivery. These mechanisms must include the effect of the circulating corticoids during pregnancy; although the corticoids were administered in the same

dosage as in the postpartum period, they may have had a prolonged survival time and antagonized the vasopressin.

One explanation favored by the authors is that some factor was present prior to delivery which was inactivating the antidiuretic principles. Support for this theory is found in experimental work which has shown that the placenta and other tissues may inactivate vasopressin *in vitro* (5, 6). Furthermore, it has been reported that inactivation of this material occurs after it has been incubated *in vitro* with the plasma of pregnant women (7). This inactivation has been shown (at least for the pressor activity of vasopressin) to be present in maternal, but not in fetal blood (8). The rate of inactivation and the optimum pH and temperature for this reaction are those characteristic of an enzymatic process. This suggests that the placenta elaborates a circulating enzyme which inactivates antidiuretic hormone.

In this case it is also of interest to note the recurrence of diabetes insipidus in the preterminal state. Recurrence has been observed in other post-hypophysectomy patients under our care, both in the preterminal period and during periods of pyrexia associated with intercurrent infec-

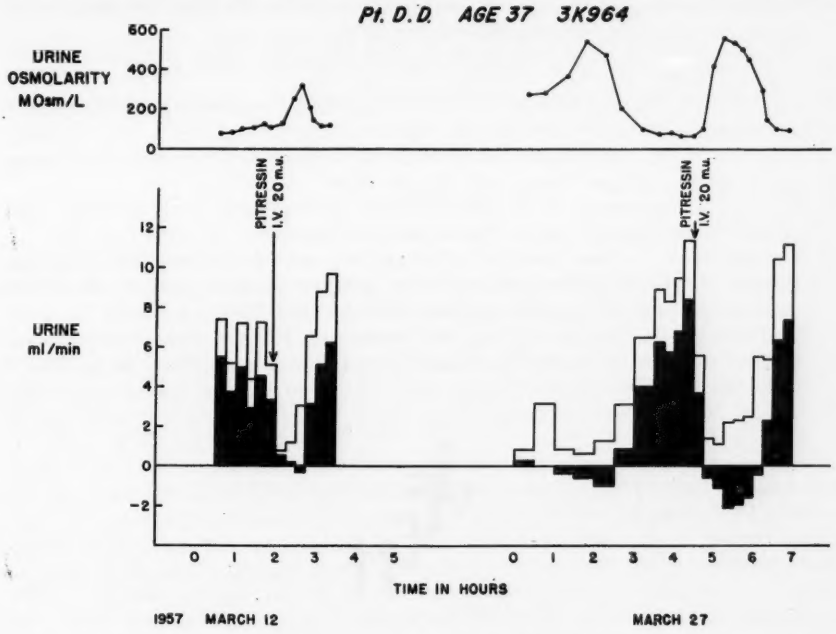

FIG. 2. Urine volumes and osmolarity during the 2 periods of study in which intravenous Pitressin was administered—in the thirty-fifth week of pregnancy and on the thirteenth postpartum day.

tions. It may be explained by the assumption that these patients have a "latent" form of diabetes insipidus which is not clinically apparent during periods without polyuria. Their endogenous secretion of antidiuretic hormone is sufficient to keep the urine volumes at normal levels under most conditions. When an increased osmolar load is presented to the kidneys, however, the polyuria and polydipsia become manifest. The increased osmolar load in this patient presumably was due to the increased catabolic activity occurring in the preterminal period and associated with the pyretic process. In contrast to the effect during the period of gestation, vasopressin therapy was potent when given at this time to control the nocturnal polydipsia and polyuria.

Acknowledgments

The authors wish to express their thanks to Dr. William B. Schwartz for his valuable help and advice in the preparation of this paper.

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Letters to the Editor

COMPLETE SEX REVERSAL: A CASE REPORT

To the Editor:

Recently cases of Turner's syndrome (gonadal dysgenesis) with a male sex chromatin pattern and the presence of some degree of ovarian elements have been reported by Hoffenberg and Jackson (1), and by Greenblatt (2). Such cases are important in the development of theories of sexual differentiation. Ashley and Jones (3) observed an infant with phallic enlargement in whom there was a similar discrepancy between chromosomal and gonadal sex. We wish to report a case in which a male chromatin pattern was found in a subject with normal ovaries and no phallic enlargement or stigmata of Turner's syndrome.

The patient was twice admitted to the hospital for diagnosis and treatment of multiple complaints. Ultimately there was well documented evidence for an intestinal malabsorption syndrome which accounted for what seemed to be initially a bizarre and unrelated assortment of symptoms and findings (4). This report will be confined to the endocrine aspects of the case.

CASE REPORT

V.H., a 13-year-old girl, was referred to the University of California Medical Center in March 1958, with the chief complaint of failure of growth and lack of sexual development. The patient was the product of an unremarkable full-term first pregnancy and her early general health had been good. Data on early growth were not available, but for eighteen months prior to entry there had been little change in height or weight. Family growth patterns were not unusual and there was a 9-year-old sister who was taller than the patient. The patient's mother was a short adult (5 feet) and had been shorter than average as a child, but her sexual maturation had occurred early, with menarche at the age of 10 years.

Physical examination of the patient on admission to the hospital revealed a girl who was small for her age and had a protuberant abdomen (Fig. 1). Her height was 4 feet 5 inches and weight 59½ pounds. Mild cheilosis and dental caries were present. There was no axillary or pubic hair, no breast development, and the external genitalia were of normal but immature appearance. Although the abdomen was protuberant there were no palpably enlarged organs. On rectal examination no internal genitalia could be felt. There were no stigmata of Turner's syndrome. The rest of the physical findings were unremarkable.

X-ray examination revealed a normal skull pattern and a retarded bone age of 10 years (chronologic age, 13 years). Thyroid function studies showed a normal serum pro-

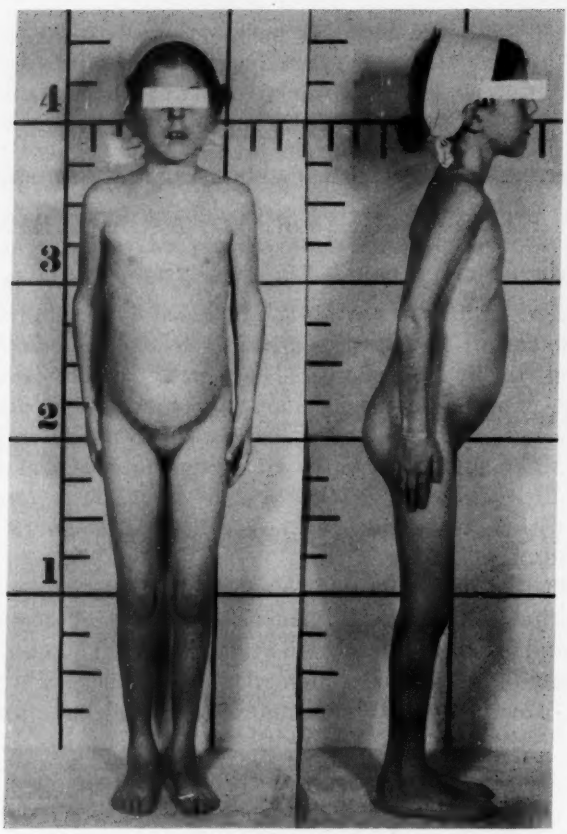

FIG. 1. Anterior and lateral views of Patient V.H., showing stunted growth and protuberant abdomen.

tein-bound iodine level, a normal I^{131} uptake before and after stimulation with thyrotropin, and a normal level of serum cholesterol and alkaline phosphatase. The urinary excretions of 17-ketosteroids and 17-hydroxycorticoids were normal before and after a standard intramuscular ACTH stimulation test.

In five 24-hour urine collections the titers were negative for gonadotropins at 2.5 and 5.0 mouse units. Examination of 6 buccal mucosal smears and 3 vaginal mucosal smears failed to demonstrate nuclear sex chromatin, *i.e.*, all showed a male pattern.

In order to determine gonadal status, exploratory laparotomy was performed. Under anesthesia the vagina was found to be narrow but of normal depth, and a small cervix was seen. Pelvic exploration (Fig. 2) revealed a small (2.5 cm.) bicornuate uterus with small but normal tubes, and bilateral gonads approximately $1 \times 1 \times 3$ cm. in size. The gonads were smooth and whitish, with small cystic areas. Wedge biopsy specimens were obtained from both poles of the left gonad, and from the central area and the lower pole of the right gonad. Histologically, all 4 biopsy specimens contained active ovarian

FIG. 2. Drawing of the pelvic structures at the time of laparotomy. Note the small bicornuate uterus and the small but apparently normal Fallopian tubes and gonads; the latter grossly resembled ovaries.

tissue with prominent cortical stroma (Fig. 3). Multiple primordial follicles were scattered throughout all areas. There was no evidence of ovulation.

COMMENT

Since the discovery of technics of sex chromatin determination, there have been many advances in the diagnosis and management of patients with sexual ambiguity. There also have been many theories concerning the normal pathways of sexual differentiation and how aberrations from these pathways occur. If nuclear sex chromatin does represent true chromosomal sex, then evidence is accruing that there must be factors relevant to sexual differentiation in addition to the established one of local fetal testicular effect. This effect, demonstrated by Jost (5) and well presented by Grumbach *et al.* (6), explains the occurrence of a negative chromatin pattern (as in a normal male) in many cases of Turner's syndrome, since the Müllerian duct system is dominant, irrespective of original chromosomal sex, when the testes have been ablated at an early stage. This concept was extended to explain many of the findings in patients with various types of intersex, but Hoffenberg and Jackson (7) have noted discrepancies which this theory does not completely answer. They hypothesize that some kind of male and female "evocators" are present as controlling agents in differentiation. Our case and the other reported cases favor the latter theory, since they cannot be explained by simple fetal testicular castration, which would not account for the ovarian tissue. They seem to indicate a bipotentiality of the primitive germ cells, and there is evidence from animal experimentation (8) to support this. Such a germinal bipotentiality would also account for the cases of Klinefelter's syndrome which exhibit nuclear sex chromatin

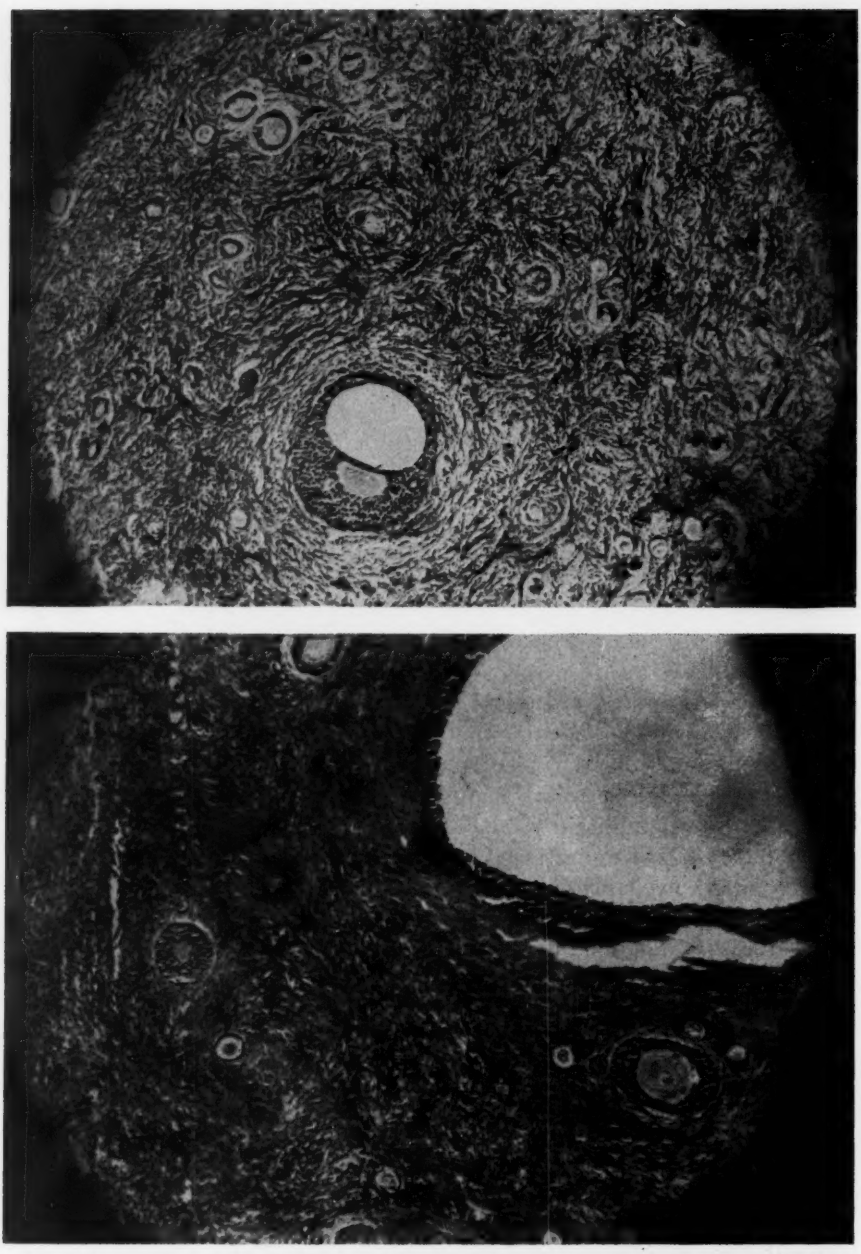

FIG. 3. Representative biopsy sections of ovaries. Note the formation of primary follicles.

(female pattern). Other than the chromosomal sex pattern and the local presence or absence of testes, the exact nature of the factors or "evocators" involved in sexual differentiation and in influencing this bipotentiality is yet unknown.

It will be of interest to see whether normal female sexual maturation occurs in our patient. If it does occur, there is the possibility that half of the ova produced will contain Y instead of the usual X chromatin. Whether fertilization of a Y ovum with a Y sperm could occur and what form the resulting organism would take is another intriguing but unanswerable question. Discovery and follow-up of further cases similar to that reported here may help to answer such questions.

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October 20, 1958

Acknowledgment

We wish to thank Dr. Warren Nelson for his help in examining the buccal and vaginal smears for sex chromatin.

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INTERFERENCE BY COPPER IN THE DETERMINATION OF SERUM BUTANOL-EXTRACTABLE IODINE*

TO THE EDITOR:

In this laboratory during 1957, the results of serum BEI (butanol-extractable iodine) determinations (1) were unexpectedly low; insidiously, recoveries fell below 33 per cent of theoretical values; no one factor could be incriminated. False low values persisted after preparation of new solutions of all reagents until Dr. Samuel Jacobson of New Haven Water Company suggested measurement of the copper content of the distilled water obtained from our 25-year-old steam still. Freshly distilled water from this source contained 0.02 part of copper per million or 2 micrograms per 100 milliliters of water even after demineralization with an anion and cation mixed bed resin cartridge. After substitution of distilled water without determinable traces of copper, BEI was recovered quantitatively from sera.

Various investigators (2–5) have mentioned distortion from trace quantities of mercury or osmium and complete blocking from silver, in the measurement of iodide by the arsenious-acid ceric-sulfate method. Obstruction by copper was not recognized in 1937 by Sandell and Kolthoff (6), but was mentioned by Smith (7) and by Kolthoff and Sandell (8) in relation to insolubility of cuprous iodide. Iodometric quantitation of copper (8–11) and interference by arsenic in such measurements (8, 11, 12) are reactions which suggested that copper would diminish the accelerating effect of iodide on reduction of ceric sulfate by arsenious acid.

The first tests to verify interference from copper were not as conclusive as had been anticipated in relation to iodide deficits when distilled water contained traces of copper. Results of these tests are presented in order to assist other investigators if trace quantities of heavy metals should insidiously lower quantitative recoveries of PBI (protein-bound iodine) or BEI.

RESULTS AND DISCUSSION

During the 1957 episode practically no iodide was found after the samples being analyzed either for BEI or inorganic iodine had been distilled. However, iodide of potassium iodide added to the colorimeter tubes in the final quantitation step was recovered more satisfactorily. Reagents and procedures were those described in our published BEI methods (1, 13), and evaporation was the usual step by which the BEI distillate was concen-

* The experimental data were obtained in a study aided by a grant from the United States Public Health Service A 392-C12.

trated. Recoveries of iodide of potassium iodide (first 4 tests in Table 1) were low and inconsistent (15 to 80 per cent) in evaporated samples as compared with unevaporated samples in colorimeter tubes.

When a trace amount of copper sulfate was added to the dilute potassium iodide solution and the mixture allowed to stand at room temperature for fourteen days, and also to the solution of arsenious acid in 0.25 *N* sulfuric acid for a few days, duplicate recoveries of iodide were reduced from an average of 13.5 to 10.5 μg. per 100 ml. (Table 1, second 4 tests). The concentration of the copper in the final quantitation tube was estimated to be 0.13 part per 100,000 or 130 μg. per 100 ml., but no tests were made as to proportions of cupric and cuprous ions. Interference from cupric sulfate in the dilute potassium iodide solution was not as consistent immediately after addition of the cupric sulfate. Thus the iodide arsenious-acid ceric-sulfate reaction was only partially blocked in spite of the fact that copper concentration from added cupric sulfate greatly exceeded the copper concentration in the 1957 "iodine-free water" (2 μg. per 100 ml.).

A particle of metallic copper was added to the potassium iodide and arsenious acid in a beaker containing about 70 ml. of water before evaporation of the solution. In these tests (Table 1, last group), reduction of ceric sulfate by arsenious acid was significantly decreased; lower recoveries were obtained in samples of higher iodide content. Copper content, based on a single analysis, was only about half that in the cupric sulfate samples. In the last 3 tests in the table, addition of copper pellets only to the colorimeter tubes for final quantitation did not lower the recoveries of added iodide.

In 1957, after iodine-free water without copper had been obtained, recoveries of iodide were still inconsistent. The final quantitation apparatus had always routinely been soaked in dilute dichromate solution. With substitution of dilute nitric acid as a cleaning agent, BEI recoveries became satisfactory. In the tests, beakers were exposed to dilute cupric sulfate, then soaked in concentrated dichromate cleaning solution and finally rinsed with distilled and iodine-free water. Only about 70 per cent of the iodine of the potassium iodide in these beakers was recovered (Table 2). Sandell mentioned reactions between copper and chromic ion (12). Zak *et al.* (4), in regard to falsely low serum PBI values in patients treated with mercurial diuretics, recommended decontamination of glassware with nitric acid. Our experience confirms their observation that nitric acid is more efficient than chromic acid for removal of heavy metals absorbed on the glassware used for BEI determinations.

Since iodide recoveries were lower in 1957 when the water contained copper, than in the later planned tests, interference by copper at early steps in the procedure cannot be denied. Copper not only forms insoluble cuprous

TABLE 1. INTERFERENCE BY COPPER IN BEI PROCEDURE ONLY IN FINAL STEPS OF QUANTITATION, FOLLOWING DISTILLATION

KI solution*		Presence of copper	Sample started in	
Age (days)†	μg. I/100 ml.		Colorimeter tube (unevap'd) (μg.I/100 ml.)	Beaker (70 ml. before evaporation) (μg. I/100 ml.)
		In distilled water (1957)		
1	10.6	0	9.3	6.9
1	10.6	0	11.9	8.4
1	5.3	0	4.7	0.8
1	5.3	0	5.3	4.0
		Cupric sulfate (1958)		
15	14.2	0		13.0
15	14.2	0		14.1
14	14.2	+‡		11.0
14	14.2	+‡		10.1
		Copper pellet§ (1958)		
1	14.2	0		13.7
1	14.2	0		14.6
1	14.2	+		11.4
1	14.2	+		9.4
0	14.2	0		14.6
0	14.2	0		14.6
0	14.2	+		11.0
0	14.2	+		11.0
1	4.3	0		4.5
1	4.3	0		4.1
1	4.3	+		3.0
1	4.3	+		3.3
18	15.4	+		10.5
17	15.4	0	15.7	
17	15.4	0	15.7	
17	15.4	+	14.8	

* Dilute potassium iodide solution containing approximately 0.05 μg. of iodide per ml.

† Dilute solutions may deteriorate after 48 kours. In these experiments the original solution was retained to eliminate variations produced by use of different solutions.

‡ Addition of cupric sulfate solution equivalent approximately to 1.3 μg. of copper per ml. of solution in final colorimeter tube.

§ From a metallic copper pellet, 0.75 μg. of copper per ml. of solution in final colorimeter tube, as estimated from only one quantitation of copper content in solution. This quantity of copper in the solution before evaporation in the beaker would be equivalent to 1 μg. of copper per 100 ml. of solution, or half the copper content of the 1957 distilled water.

TABLE 2. RECOVERIES OF POTASSIUM IODIDE AFTER DIFFERENT CLEANING METHODS FOR COLORIMETER TUBES

Iodide (μg./100 ml.)	Before final washing, tubes soaked 30 min. in	
	Dil. nitric acid (μg. I/100 ml.)	Dil. chromic acid (μg. *I*/100 ml.)
Year 1957		
13.8	14.1	5.6
13.8	14.7	4.6
13.8	13.3	11.6
Year 1958		
5.0	5.2	3.8
5.0	5.3	3.8

iodide but also forms complexes with thyroxine, thyronine, diiodothyronine, triiodothyronine (14), and arsenic salts. Both in the distillation procedure and in the final quantitation of iodide with ceric sulfate, arsenious-acid solutions are used. Suspicion that these arsenious-acid solutions were vulnerable to traces of copper was derived from observations that in samples reduced with arsenious acid during distillation, recoveries ranged between 0 and 33 per cent in contrast to recoveries of 50 per cent in samples carried through only the steps subsequent to distillation.

In relation to interference by heavy metals, silver nitrate and cupric sulfate behave differently. Kontaxis and Pickering (5) recently published a method for measurement of serum BEI concentration, which is dependent on cessation of the arsenious-acid ceric-sulfate iodide reaction by addition of silver nitrate after a definite interval of the reaction. In a few tests with our concentrations of arsenious acid and ceric sulfate, the addition of cupric sulfate (after the reduction of ceric sulfate was progressing) did not alter the rate of color fading. Kontaxis and Pickering discussed in detail the mechanics of the arsenious-acid ceric-sulfate reaction and interference by heavy metals.

SUMMARY

Spotty, unreliable recoveries of iodide of potassium iodide occurred when a trace of copper sulfate was added to solutions after the distillation stage of the serum BEI procedure. Greater reductions of measurable BEI had been observed when distilled iodine-free water contained traces of copper.

Dilute nitric acid is a more effective decontaminating agent than dilute chromic acid for removal of heavy metals from the glass apparatus used in the BEI technique.

The occurrence of low and inconsistent values for serum PBI or BEI con-

centrations should alert the clinical chemist to the possibility of contamination from traces of a heavy metal such as copper or other impurities in reagents or in "iodine-free water."

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September 24, 1958

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The Endocrine Society

THE 1959 ANNUAL MEETING

The Forty-First Annual Meeting of The Endocrine Society will be held in the Haddon Hall Hotel, Atlantic City, New Jersey, Thursday, Friday, and Saturday, *June 4, 5 and 6, 1959.*

The Chairman of Local Arrangements is Dr. Matthew Molitch.

Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily, and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 5, at 7:30 P.M. preceded by cocktails at 6:30 P.M.

All members are urged to make their hotel reservations immediately. The Chalfonte-Haddon Hall Hotel will hold 300 bedrooms for members until May 1, 1959, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations now directly with the hotel, advising them of time and date of arrival and departure. Avoid disappointment.

Final program, membership card and advance registration forms will be sent on May 1, 1959 to members whose current dues have been paid.

THE 1959 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

FRED CONRAD KOCH AWARD

During the past year a substantial legacy has been bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is to be the highest honor of The Endocrine Society and is to be represented by a medal that is to be known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an in-

dividual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award will replace the Medal of The Endocrine Society which was established in 1954 and presented to Dr. Carl R. Moore in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957 and Dr. I. L. Chaikoff in 1958. The Medal of the Endocrine Society replaced the E. R. Squibb Award which was formerly the highest honor bestowed by the Society. Past recipients of the Squibb Award were Dr. George W. Corner in 1940, Dr. Philip E. Smith in 1941, Dr. Fred C. Koch in 1942, (no award in 1943), Dr. E. A. Doisy in 1944, Dr. E. C. Kendall in 1945, Dr. Carl G. Hartman in 1946, Drs. Carl F. and Gerty T. Cori in 1947, Dr. Fuller Albright in 1948, Dr. Herbert M. Evans in 1949, Dr. C. N. H. Long in 1950, Dr. J B. Collip in 1951, Dr. James H. Means in 1952 and Dr. David Marine in 1953.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr. Monte A. Greer. Prior to 1952 the Award was $1,200. It has now been increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.

3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

ELEVENTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE UNIVERSITY OF CALIFORNIA SCHOOL OF MEDICINE AND DEPARTMENT OF CONTINUING MEDICAL EDUCATION

SAN FRANCISCO, CALIFORNIA

November 9 through 13, 1959

For information concerning the program and registration, write to *Seymour M. Farber, M.D.*, Assistant Dean in charge of Continuing Medical

Education, University of California Medical Center, San Francisco 22, California.

The registration fee is $100. Residents and Fellows will be admitted for a reduced fee of $30. Those wishing to reserve rooms should communicate directly with the Whitcomb Motor Hotel, 8th and Market St., San Francisco.

FIRST INTERNATIONAL CONGRESS OF ENDOCRINOLOGY

The First International Congress of Endocrinology will be held in *Copenhagen, Denmark, July 18–23, 1960.*

United States members of the International Executive Committee are Dr. Warren O. Nelson and Dr. Leo T. Samuels. Dr. Gregory Pincus is Chairman of the Program Committee. The tentative program consists of ten symposia covering the general field of endocrinology plus round-table discussions and a number of submitted papers. Those desiring to submit papers should immediately contact Dr. Gregory Pincus, Worcester Foundation, Shrewsbury, Massachusetts. There will be a number of interesting scientific and technical exhibits.

In order to facilitate travel arrangements to the Copenhagen Convention, the Council of The Endocrine Society at its San Francisco meeting designated THOMAS COOK & SON as our authorized travel agents. They will in turn offer especially designed All Expense Paid Tours to Europe, including hotel reservations for the convention, as well as offering independent itineraries for any of our members who desire to travel separately by air or by steamship.

Registration is open to anyone interested in endocrinology at an approximate fee of $28 U.S. Members of the families of those attending may register as associates at an approximate fee of $14 U.S., fee to be paid at the time of registration or in advance to the American Express Company, which has been designated by the Executive Committee of the International Congress as official financial and travel agent. There will be a number of social functions and other entertainment provided for all registrants.

As you all know, European travel is unusually heavy during July and it is suggested that those contemplating attending the Congress should start making tentative reservations at the earliest possible moment by filling in and returning the card recently mailed to members, to THOMAS COOK & SON's special representative—Harry E. Kornbaum, Transportation Chairman, Rainbow Travel Service, 2817 Classen Boulevard, Oklahoma City 6, Oklahoma, who will correspond directly with each member about

his individual travel arrangements to and from the Convention, at the same time keeping our National Officers fully informed of all travel arrangements of its members. Completion of the card CARRIES NO OBLIGATION OR COMMITMENT ON YOUR PART. Send it in today for the complete travel arrangements for our Society.

The general expenses of a meeting of this magnitude are quite large and The Endocrine Society has made a substantial contribution from its treasury for organizational purposes. U.S. pharmaceutical firms have also contributed very generously. However, sufficient funds are not as yet available to assure the success of this meeting; therefore, the committee suggests that each of our members make voluntary contributions to this fund. Please make your checks payable to: The Endocrine Society—First International Congress of Endocrinology. Mark it *Contribution* and mail it to the office of the Secretary-Treasurer, 1200 North Walker Street, Oklahoma City 3, Oklahoma.

The American Goiter Association

THE 1959 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the Drake Hotel, Chicago, Illinois, on *April 30, May 1 and 2, 1959.*

1959 LAURENTIAN HORMONE CONFERENCE

The 1959 Laurentian Hormone Conference of the AAAS will be held at *Mont Tremblant Lodge, Mont Tremblant, Quebec,* during the period *August 30–September 4.* Investigators interested in attending this Conference should make application to the Committee on Arrangements of the Laurentian Hormone Conference, 222 Maple Avenue, Shrewsbury, Massachusetts, at an early date and in any event *no later than May 11, 1959.* A conference rate of $13.00 per day per person is extended to all invited participants. Since the number of our participants is necessarily limited by available accommodations, all applications are screened and invitations to attend are issued by the Committee on Arrangements by June 12. The following program has been arranged.

PROGRAM

I. Mechanism of Hormone Action

Participation of steroid hormones in the enzymatic transfer of hydrogen.
Paul Talalay and H. G. Williams-Ashman, The Ben May Laboratory, University of Chicago.
Sunday evening, August 30

An enzymatic basis for the physiologic function of estrogens.
Claude A. Villee and Dwain D. Hagerman, Harvard University Medical School, Boston.
Sunday evening, August 30

Effects of steroid hormones on some coenzyme mediated enzymatic reactions.
Lewis L. Engel and Jesse F. Scott, John Collins Warren Laboratories of the Huntington Memorial Hospital of Harvard University, Massachusetts General Hospital, and Departments of Biological Chemistry and Anatomy, Harvard Medical School.
Monday morning, August 31

The mechanism of action of some estrogenic hormones on metabolism.
Kenneth W. McKerns, Edwin Kaleita and Paul H. Bell, Lederle Laboratories, American Cyanamid Company, Pearl River, New York.
Monday morning, August 31

The role of cyclic adenylic acid in hormone action.
Robert C. Haynes, Jr., E. W. Sutherland, Jr., and T. W. Rall, Western Reserve University School of Medicine, Cleveland.
Monday morning, August 31

Cell permeability and hormone action.
O. Hechter and G. Lester, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts.
Monday evening, August 31

II. Peptide Hormones

The immunological assay of human pituitary growth hormone.
Charles H. Read and George T. Bryan, State University of Iowa, University Hospital, Iowa City.
Tuesday morning, September 1

Studies on erythropoetin.
W. F. White, Clifford W. Gurney and Eugene Goldwasser, Armour and Co. and Argonne Cancer Research Hospital, Chicago.
Tuesday morning, September 1

Hormonal activity of active polypeptides.
Hector Croxatto and Livio Barnafi, Catholic University, Santiago, Chile.
Tuesday evening, September 1

III. Thyroid Hormones

Studies on the chemistry and bioassay of thyrotropin from bovine pituitaries and transplantable pituitary tumors of mice.
Robert W. Bates and Peter G. Condliffe, National Institutes of Health, Bethesda
Wednesday morning, September 2

The transport of thyroid hormone and its relation to its metabolic fate.
Sidney Ingbar, Thorndike Memorial Laboratory, The Boston City Hospital, Boston
Wednesday morning, September 2

Re-evaluation of some concepts of iodine metabolism.
L. Van Middlesworth, The University of Tennessee, Memphis, Tennessee.
Thursday morning, September 3

IV. Hormones and Metabolism

Food and temperature.
John R. Brobeck, University of Pennsylvania School of Medicine, Philadelphia.
Thursday morning, September 3

Influence of hormones on adipose tissue as a center of fat transport.
H. E. Wertheimer, The Hebrew University—Hadassah Medical School, Jerusalem, Israel.
Thursday evening, September 3

Hormonal control of protein and fat metabolism in the pancreatectomized rat.
Robert O. Scow and Sidney S. Chernick, National Institutes of Health, Bethesda.
Friday morning, September 4

Hormonal regulation of hepatic carbohydrate metabolism.
J. Ashmore, Indiana University Medical Center, Indianapolis, Indiana; *George Cahill, Jr.*, New England Deaconess Hospital, Boston; and *A. Baird Hastings*, Scripps Metabolic Clinic, La Jolla, California.
Firday morning, September 4

INTERNATIONAL CONGRESS OF GASTROENTEROLOGY

The 6th Meeting of the Association of the National European and Mediterranean Societies of Gastroenterology, organized by the Association of Dutch Gastroenterologists, will be held in Leiden, the Netherlands, from *April 20 to 24, 1960.*

The main themes of the conference will be: 1) Pathology of the small intestine; 2) Hepatitis, cirrhosis hepatis and their possible connection.

Titles for papers together with a summary of not more than 200 words should be sent not later than *August 1, 1959*, to Dr. B. K. Boom, Congress Office, Department of Gastroenterology, University Hospital, Leiden, the Netherlands.

For further information and registration apply to the Congress Office of the 6th Meeting of the Association of the National European and Mediterranean Societies of Gastroenterology, Department of Gastroenterology, University Hospital, Leiden, the Netherlands, or the Secretary of the national societies of gastroenterologists.

BIOLOGICAL PHOTOGRAPHIC ASSOCIATION

The first Midwestern Sectional Meeting of the Biological Photographic Association will take place in Iowa City, Iowa, *April 24 through 26* at the University of Iowa. Distinguished photographic specialists in medicine, dentistry, biology, and other sciences will attend. There will be striking displays of clinical and specimen photography, photomicrography and natural science. For further information write to Mr. Carroll H. Weiss, Director of Professional and Press Publicity, 160 Riverside Drive, New York 24, N. Y.

CHILDREN'S HOSPITAL OF PHILADELPHIA

A series of short *Refresher Courses* will be given by the Children's Hospital of Philadelphia and by the Graduate School of Medicine, University of Pennsylvania, as follows: 1) Pediatric Advances, *May 25–29, 1959*, tuition $115.00; 2) Practical Pediatric Hematology, *June 1–5, 1959*, tuition $125.00; 3) Postgraduate Course in Practical Pediatrics for physicians in Pennsylvania, New Jersey and Delaware, Wednesday afternoons, *March 18–June 4*, tuition $100.00. Inquiries should be addressed to Irving J. Wolman, M.D., Director of Post-Graduate Education, the Children's Hospital of Philadelphia, 1740 Bainbridge Street, Philadelphia 46, Pa.